CONCERNING ADENOMATA ORIGINATING FROM THE ISLANDS OF LANGERHANS.*

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PLATE LXXIX.

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Although primary neoplasms of the pancreas are fairly uncommon, medical literature discloses no lack of variety in the types of pancreatic tumor which have been reported. Primary carcinoma of the pancreas is not rare; and a few cases of sarcoma are recorded. Benign tumors of the pancreas, although exceedingly rare, have also been described. Cesaris-Demel (1) has reported an adenoma which he thinks originated from the pancreatic acini. Biondi (2) encountered a fibro-adenoma in the pancreas, the arrangement and morphology of its cells closely resembling those of the pancreatic ducts. Multilocular cystomata of the pancreas have been reported by Garrigues (3), Heaton (4), and others. A "myxomatous angioma" of the pancreas is recorded by Baudach (5).

All of these neoplasms mentioned above are supposed to have originated from either the secretory structures or the stroma of the pancreas. I have been able to find in the literature only three references to tumors of the islands of Langerhans.

In 1902, Fabozzi (6) reported five cases of carcinoma of the pancreas, all of which he believed had arisen from the islands of Langerhans. Reitmann (7) and Helmholtz (8) have both criticized Fabozzi's work and have questioned his conclusions. Judging from the descriptions of his cases, the diagnosis of carcinoma of the pancreas was in every instance correct. His drawings, however, by no means convince me that the tumors had originated from islands of Langerhans. The protocols of one or two of the cases lead me to suspect that the pancreatic tumor was a metastatic nodule following carcinoma of the gall bladder. At all events, Fabozzi's theory that they developed from islands of Langerhans can not be accepted until more evidence is forthcoming.

As far as I am aware, only two benign tumors purporting to have originated from the islands of Langerhans have been described. The first of these two cases

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was that reported by Nicholls (9) in 1902. The tumor, which was not larger than a marrow-fat pea was situated on the anterior surface of the tail of the pancreas. It had a tawny yellow color, was well circumscribed, rather soft, and exuded a little blood. Microscopically, the tumor appeared as an oval area in the pancreatic substance, measuring 3 by 2.5 mm. It was enclosed by a delicate fibrous capsule. The tumor was composed of elongated and irregular masses of cells bounded by anastomosing bands of fibrous tissue. In a few cases a tendency to form lumina was observed, but a small mass of connective tissue could almost invariably be made out in the center. Minute blood-vessels were found throughout the delicate stroma. The tumor cells were polyhedral and short columnar in form. Their cytoplasm was granular and eosinophilic. The nuclei were vesicular, each containing several dots of chromatin. The cells were slightly smaller than those of the pancreatic acini. Nicholls considered this tumor to be a simple adenoma originating from an island of Langerhans.

Helmholtz's case was that of a negro, sixty-five years old, who died of chronic cardiovascular disease. At the autopsy the pancreas was normal except for a small nodule 5 by 3 by 5 mm. which was discovered accidentally in the substance of the gland. The tumor was dull gray, finely granular, and sharply circumscribed by a thin capsule. Microscopically, the nodule was found to be composed of masses of polyhedral cells, supported in a delicate mesh-work of fibrous tissue. In some places these cell masses were solid; in others, lumina were seen, about which cells of a columnar type were arranged. The slender trabeculæ that supported the groups of cells contained capillaries. The tumor cells resembled in every way those composing an island of Langerhans. They were arranged in anastomosing cords in intimate relationship with the capillaries, possessed an acidophilic cytoplasm and vesicular nuclei with large chromatin bodies. After studying this tumor, Helmholtz was convinced that it was an adenoma, having its origin in an island of Langerhans.

DESCRIPTION OF CASE.

The object of this paper is to record a third tumor of the type reported by Nicholls and by Helmholtz.

It occurred in the pancreas of a man sixty-three years old, who was admitted to the second surgical service of the Presbyterian Hospital on October 26, 1908. His family and personal history were of no interest. For a year previous to his admission he had not been in good health. He was now suffering from pain in the right lower quadrant of the abdomen, constipation, anorexia, loss of weight, and some edema of the feet and ankles.

Physical Examination.—The heart and lungs were normal; the abdomen soft and symmetrical. To the right of the umbilicus a mass 2 by 3 inches could be felt, somewhat tender and fixed to surrounding parts. The skin over the tumor was freely movable.

The patient lost ground rapidly. On November 1 he passed about ten ounces of blood per rectum. The urine contained no albumen or sugar. The patient died on November 7.

At the autopsy, performed twenty-four hours after death, the body was found to be that of an emaciated old man. The abdomen only was examined. The liver and spleen were normal. The kidneys were finely granular and had thin cortices. The prostate was enlarged. In the ascending colon there was found a large tumor which extended up to the hepatic flexure. It was annular in shape. The outer surface was smooth and hard; the inner surface was papillomatous, with large necrotic ulcerated areas, and covered with mucus. The lymph glands in the mesocolon were not enlarged. Microscopical sections from the tumor showed it to be an adenocarcinoma.

The pancreas was firm and pale. The pancreatic ducts were patent. At one point in the caudal portion, near the surface, but completely surrounded by pancreatic tissue, there was a small spherical tumor, the size of a French pea. On section, the cut surface measured 4 by 3.5 millimeters. The tumor was well circumscribed and enclosed by a delicate capsule within which it was freely movable.

The tissue was hardened in Zenker's fluid and imbedded in paraffin. Part of the tumor was cut in serial sections and stained with hematoxylin and eosin and with toluidin blue and eosin. The connective tissue stains of Van Gieson and Mallory were also employed.

Microscopically, the tumor, under low magnification (figure 1), is seen to be sharply demarcated from the surrounding pancreatic tissue by a delicate capsule of fibrous connective tissue. It is composed of irregular elongated masses of cells, which lie in a rather coarse mesh-work of hyaline stroma. No ducts are found in connection with the tumor.

Under high magnification (figure 2), the tumor cells are small and poorly differentiated from one another. They appear, however, to be polyhedral or low cuboidal in shape. As a rule, they show no definite arrangement, being packed loosely together several layers thick. In some places, however, a single layer of cuboidal cells lines a sort of acinus which may or may not contain blood. These

cells rest directly on the hyaline stroma which supports them. The cytoplasm of the cells contains coarse eosinophilic granules. Their nuclei are large and vesicular and usually show several chromatin bodies. No mitotic figures are seen.

The stroma has been converted for the most part into a homogeneous hyaline material which stains deeply with eosin. Anastomosing bands of this hyaline substance separate and support the alveoli. In some places the stroma retains a somewhat fibrillar structure and a few spindle-shaped nuclei are made out. Small capillaries can be seen coursing through the hyaline trabeculæ. Many of them have obviously been destroyed by the hyaline changes; in numerous instances, however, the endothelial lining is well preserved in spite of the hyaline degeneration about it. The occasional occurrence of diffuse hyaline patches, in which atrophic and partially hyaline alveoli may sometimes be seen, indicates that the parenchyma as well as the stroma has been involved in the degenerative changes. Altogether the appearance of the tumor suggests a greatly hypertrophied island of Langerhans with hyaline changes analogous to those seen in diabetes.

In sections stained with toluidin blue and eosin, the tumor cells are found to be free of zymogen granules. They take a light pinkish stain similar to that of the cells of an island of Langerhans or of the duct epithelium. In sections stained by Van Gieson's and by Mallory's connective tissue stains, the tumor cells stain as epithelial rather than as connective tissue structures. The hyaline stroma stains pink with Van Gieson's and dark blue with Mallory's stain.

The pancreatic tissue about the tumor is somewhat compressed. A thin fibrous capsule separates the glandular acini from the tumor cells, but in some places little or no capsule is present and the two types of cells lie in immediate contact with each other. No definite transitions from the one form into the other can be established, though structures suggestive of such a change occasionally occur. In general, the pancreatic acini appear entirely normal. There has been no increase in the interstitial connective tissue.

In view of the resemblance which the tumor bore to an island of Langerhans, these structures were examined with particular inter-

est. They are quite numerous and in some instances appear perfectly normal. The greater number of them, however, exhibit a well marked hypertrophy of the so-called "columnar" type, the insular cells showing, instead of their usual order, an arrangement like that of simple columnar epithelium. Loops and processes of these epithelium-like cells project out into the surrounding tissue, tending to give the island an irregular contour. Some of the loops resemble acini, but their lumina generally contain capillaries. A few of the smaller islands of this type are seen to be outgrowths from the small ductules, with which they still maintain a connection. The larger islands are usually disconnected from the ducts, but in some places are continuous with adjacent acini.

DISCUSSION.

In comparing these three tumors it will at once be seen that while they show slight variations, they present many points in common. All three occurred in the substance of the pancreas. On gross examination, they were almost identical in size and general appearance. Microscopically, they were all sharply circumscribed and enclosed by fibrous capsules. In structure they were quite similar, being composed of irregular elongated masses of cells, supported in a delicate vascularized fibrous stroma. In all three of them the cells showed in places a columnar form and tended to arrange themselves into acini, in this respect simulating the so-called "adenomatous" or "columnar" hypertrophy of the islands of Langerhans. In all three instances, the cytoplasm of the tumor cells was coarsely granular and eosinophilic and their nuclei vesicular, with several chromatin bodies. On the basis, therefore, of this similarity, we may reasonably conclude that the three tumors had the same origin.

Regarding the origin of the tumors, Nicholls points out that they must of necessity arise from one of three structures: (1) the epithelium of the ducts, (2) the pancreatic acini, or (3) the islands of Langerhans. Adenomata which have been described as originating from the ducts are composed, like other tumors of duct origin, of columnar epithelium and exhibit more or less tendency toward the formation of atypical ducts with true lumina. The tumors under

discussion, however, are composed chiefly of polyhedral cells, arranged in solid cords and masses and forming no true lumina. On the other hand, if these tumors had sprung from the pancreatic acini, one would expect to find in them some similarity to acini in their structure and in the arrangement of cells. Such resemblance, however, is lacking. The tumor cells, as has been stated, show only an occasional tendency to form lumina or acini and are, moreover, entirely free from zymogen granules. When the cells are columnar and arranged like acini, the appearance is that of a hypertrophied island rather than of secreting acini. When stained by toluidin blue and eosin, Van Gieson's, or Mallory's stain, the tumor cells correspond in every way with those of an island of Langerhans. The elongated masses of cells, supported in a vascular framework of fibrous tissue are exactly analogous to the cell masses composing an island of Langerhans. Moreover, the cells themselves with their coarsely granular, acidophilic cytoplasm and vesicular nuclei rich in chromatin force one to the opinion that the tumors had their origin in an island of Langerhans. It is also significant that in my case the tumor showed extensive hyaline degeneration. This change, when it does occur in the pancreatic parenchyma, is usually seen in the islands of Langerhans.

It is safe to conclude, therefore, that all three of these tumors developed from islands of Langerhans. The question next to be answered is: Are these tumors true neoplasms, or are they merely giant islands? In other words, have we to do with genuine adenomata or simple regenerative hypertrophy of the islands? The problem which confronts us is not a new one. In the breast, in the liver, in the prostate, and even in mucous membranes, the distinction between adenoma and regenerative hypertrophy is at times difficult or impossible to make. In the pancreas, regenerative hypertrophy of the islands of Langerhans has been described in cases of diabetes by Reitmann (7), by MacCallum (10), and by me (11). More recently, Weichselbaum (12) and Kyrle (13) have reported hypertrophy of the islands in chronic pancreatitis and in experimental lesions of the pancreas. According to these authors, not only do existing islands hypertrophy, but a new formation of islands takes place from the ducts, the new islands budding off from the duct

epithelium very much after the manner of island formation in fetal life.

There was no history of diabetes in any of the three cases under discussion. In none of them was there evidence of chronic pancreatitis. In the case, however, which I have reported, it is significant that the islands show extensive regenerative hypertrophy. Their appearance has already been described. Many of them are considerably larger than normally and their cells tend to arrange themselves in single rows, with large vesicular nuclei centrally located. The cells are poor in granules and definitely columnar. The columns are separated by capillaries. In some of these islands a connection can be demonstrated, on the one hand, with the small ducts, and on the other, with the adjacent acini. The former connection is more apt to be seen in the small newly forming islands; the latter, in the hypertrophied islands. A hypertrophied island of this type suggests in a general way an adenoma; in fact the change was originally described by Reitmann as "adenomatous" hypertrophy.

In my case, at any rate, there is evidence that the islands had been put to a strain, and that in response to this strain they had undergone hypertrophy. The fact that the tumor showed hyaline degeneration rather suggests that diabetes, the commonest cause of both hyaline degeneration and hypertrophy of the islands, had been instrumental in this case. At the time the patient was in the hospital, however, there was no sugar in his urine. It is possible that by the hypertrophy of the islands his tolerance for glucose was increased to such a point that sugar disappeared spontaneously from the urine. I recall one other case in my experience where there was no history of diabetes but where the islands showed slight hyaline changes.

In a previous study of the pancreas in diabetes (11), I encountered a giant island, not so large as the tumor just reported, but comparable to it.

The case was that of an old man, seventy-five years of age. He had had diabetes for fifteen years, and finally died of pneumonia. At the autopsy, the pancreas was infiltrated with fat. Microscopical sections revealed an unevenly distributed new growth of the interstitial tissue. In places the sclerosis was quite marked. The islands were hypertrophied and were often bunched together

in patches of dense fibrous tissue. One very large island was found which measured 1.8 by 1.6 mm. in area. It was readily seen with the naked eye.

Microscopically, the large island is surrounded by a capsule of fibrous tissue which separates it entirely from the surrounding pancreatic tissue. In two or three places, small processes of island tissue project into the capsule, but elsewhere the border is regular. In their arrangement and structure, the cells of the island in part resemble those of a normal island, but in many places, especially in the outgrowths into the capsule, the island has taken on an adenomatous appearance, the cells being of the columnar type and arranged in single rows which tend to form loops or pseudo-acini. It is noticed that the secreting parenchyma is autolyzed while the cells of the island are still well preserved.

In another diabetic pancreas I found an island measuring 0.9 by 0.7 mm. in diameter. This island also exhibited the columnar type of hypertrophy. In several other cases of diabetes, islands which measured 0.4 to 0.5 mm. in diameter were encountered.

If we arrange the three tumors and the giant islands in a column with reference to their dimensions, an interesting fact is brought out:

Helmholtz's tumor	5.0 by 5.3 mm.
Cecil's tumor	4.0 by 3.5 mm.
Nicholl's tumor	3.0 by 2.5 mm.
Islands in diabetic pancreas (case XXVI)	1.8 by 1.6 mm.
Islands in diabetic pancreas (case XXIX)	0.9 by 0.7 mm.
Diameter of islands found in other cases of diabetes	0.4 to 0.5 mm.
Diameter of average normal island	0.1 to 0.2 mm.

On examining these figures it will be seen that it is almost impossible to draw a line between the tumor class and the giant island class, so gradual is the gradation in size.

The relation of these tumors to the surrounding tissue is also of interest. All three of them had capsules, but in my own case, the capsule was thin, and in a few places entirely absent. At these points the tumor cells lay in immediate contact with the glandular acini. I could find no definite transitions from acinar to tumor cells, though several suspicious forms were seen. The existence of islands of this size, whether they be neoplasms or merely hypertrophied forms, constitutes, I think, another argument in favor of the independent structure and functions of the islands. It is hard to see how exhausted acini could produce such remarkable pictures.

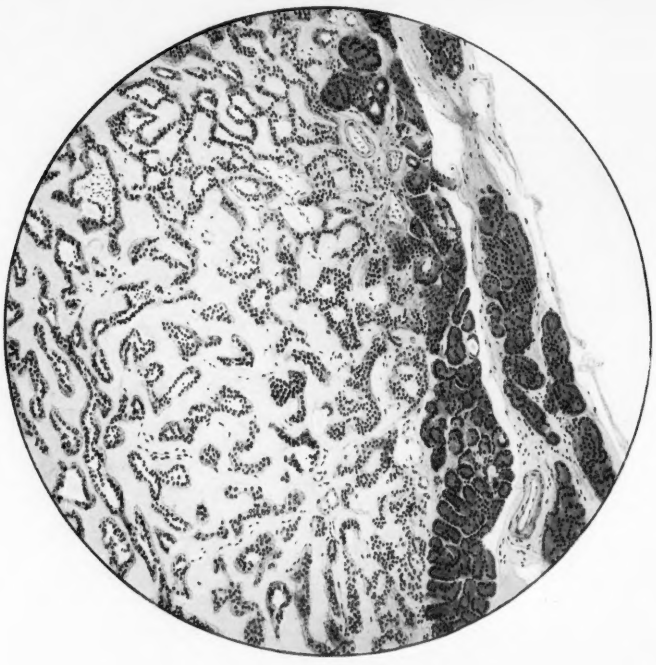

FIG. 1.

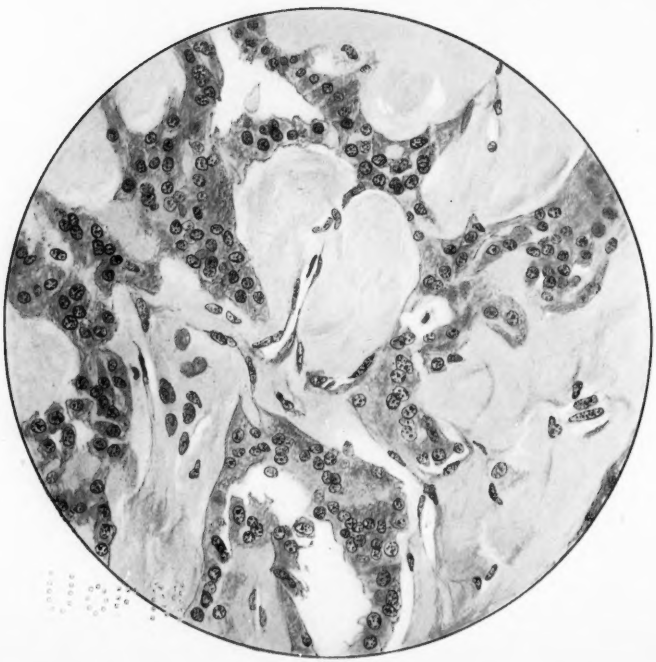

FIG. 2.

SUMMARY.

Small encapsulated tumors of the size of peas, or even smaller, are occasionally found in the pancreas. These tumors are benign, and have a structure similar in all respects to that of an hypertrophied island of Langerhans. The distinctions between adenomata and forms of regenerative hypertrophy, sometimes so difficult to make in the breast, prostate, liver, and other organs, is not easily drawn in the present case. The fact that one may encounter all gradations in the size of islands, from that of the normal island to that of the tumors, suggests that it is more reasonable to consider such tumors merely as hypertrophied islands rather than as adenomata originating from islands.

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EXPLANATION OF PLATE LXXIX.

FIG. 1. Part of the tumor, showing adjacent pancreatic parenchyma. Low magnification. (Leitz objective No. 3; ocular No. 4.)

FIG. 2. Part of the tumor under high magnification. (Leitz objective No. 7; ocular No. 4.)

THE RELATION OF THE REACTIVE STROMA FORMATION TO THE TRANSPLANTABILITY OF THE CANCERS OF THE WHITE RAT.*

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PLATES LXXX–LXXXIII.

Every malignant growth, whether carcinoma or sarcoma, consists of two parts: parenchyma, composed of cancer cells; and a connective tissue ground work, or *stroma,* which is distributed around and between the cells of the parenchyma. The true significance of the latter structure is still under discussion.

Ribbert (1) considers the stroma of paramount importance in the genesis of cancer. He believes that every normal tissue cell possesses an inherent power for unlimited proliferation, and may change into a cancer cell. The only condition for this transformation is a preliminary change in the surrounding connective tissue, which impairs the normal association of the cell with the rest of the organism. Bonney (2), in a recent investigation on the connective tissue in carcinoma, reaches very similar conclusions, *i. e.,* that the formation of carcinoma is always preceded by a precarcinomatous state, which consists in various inflammatory processes in the surrounding tissues. This precarcinomatous process assists the progress of the growth of the tumor. According to these investigators, the changes in the connective tissue are the primary etiological factors in the formation of cancers.

Hansemann (3), Heidemann (4), Borst (5), and many other pathologists, on the other hand, deny the great importance of the connective tissue for the formation of cancer. According to the view of these writers, the main functional part in cancer is the parenchyma, and the stroma is only formed subsequently under the influence of the cancer cells. This opinion is based on the following grounds:

The beginning of a new growth is not always accompanied by changes in the surrounding connective tissue. Furthermore, many characteristics in the morphological appearance of malignant tumors indicate that the connective tissue

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does not influence the proliferation of the cancer cells, but the latter, on the contrary, induce in some manner the formation of a connective tissue stroma. The amount of connective tissue between groups of cancer cells varies greatly in the different tumors. It is minimal in sarcoma, small in medullary carcinoma, and very large in scirrhous carcinoma. In the majority of cases, the connective tissue in a metastasis of a malignant tumor retains its morphological characteristics. This factor can be explained only on the basis of a *specific* influence of the cancer cells upon the surrounding connective tissue. Further proof of the specific influence of the cancer cells upon the connective tissue can be found in the fact that certain cancers act only on specific forms of connective tissue. While the stroma of a cancer usually consists of newly formed fibrous connective tissue and blood vessels, von Recklinghausen (6) and Askanazy (7) have shown that, in the bone metastasis of the carcinoma of the prostate, the cancer cells induce a formation of new bone tissue. Ehrlich (8) and Gierke (9), in their studies on hemorrhagic tumors of the white mice, noted that these tumors on every subsequent implantation produced in the new host a stroma formation consisting mainly of blood vessels.

These brief preliminary considerations show that the study of the connective tissue of malignant tumors is important, not only from the standpoint of morphological differentiation, but also for the elucidation of many factors in the growth and genesis of cancer.

The experimental study of the transplantable cancers of white mice and rats offers a better field for the investigation of the subject than the mere anatomical study of human cancer.

Jensen (10) indicated in his first publication the possibility that the new stroma is formed by the host. Bashford and his collaborators have paid a great deal of attention to this phase of the subject in their studies on cancers of the white mice. Bashford, Murray, and Cramer (11) have shown that when a piece of a mouse carcinoma is inoculated into another animal the implanted stroma becomes necrotic, and a new connective tissue stroma is formed by the host. According to Bashford, the stroma and vascular structures are a reaction on the part of the successive hosts, whereby the parenchyma is nourished and supported.

This stroma reaction of the host is perfectly specific for every tumor, *i. e.*, in all the subsequent inoculations the stroma retains the structural characteristics of the primary tumors. On the other hand, when two tumors are inoculated into the same animal, the host provides for each tumor a stroma of a different structure identical morphologically with the structures of the stroma of the original tumors used for inoculation. The same is still more apparent in the mixed tumor which appears in the white rat as a result of an injection of an emulsion of sarcoma and carcinoma cells. In the

same tumor one notices a minimal stroma formation in the parts showing the structure of sarcoma, and a great deal of stroma in the carcinomatous parts of the tumor.

Thus it is shown experimentally that the connective tissue stroma of the transplantable tumors of the white mice and rats is supplied by the new host under the influence exerted by the implanted cancer cells.

In his studies on the same subject, Russell (12), a collaborator of Bashford, goes still further in his conclusions, and tries to show that the growth of a cancer inoculated into a new host, depends upon the ability of the latter to supply the connective tissue stroma. His investigation consisted in a microscopical study of the "early stages" of tumor grafting in normal and immune animals. Small particles of tumor tissue were inoculated with a trocar into the subcutaneous tissue. After varying periods of six, twelve, twenty-four hours, etc., the grafts were removed and examined microscopically.

The results showed that while during the first two days the changes in the graft are similar in normal and immune animals, a great difference is noticed five or six days after inoculation. In the normal animal there appears an active division of the connective tissue cells of the host, leading to the development of a cellular reaction tissue, which penetrates between the islets of parenchyma. This connective tissue stroma formation is accompanied by a rich development of new blood vessels. In the immune animal, on the other hand, there is no active proliferation of the host fibroblasts, nor is there any development of new capillaries, and the new host fails to supply a vascular stroma.

This absence of "the specific stroma reaction" in immune animals is due, according to Russell and Bashford, to the suppression of the chemiotatic properties of the cancer cells. This suppression robs the cancer cells at the same time of their powers of assimilation and growth, and to this phenomenon Bashford ascribes a very general importance in the explanation of the condition of immunity, or resistance to growth of an inoculated cancer. To put it in his own words: ". . . a refractory condition may indeed not have anything to do with an action against the cancer cells, but, on the contrary, be due to an alteration of the connective tissue of the host, which hinders it from supplying the necessary connective tissue reaction."

The question whether the success of tumor transplantation in the white mouse or rat is due to the different reactions of the tissues of these animals to the implantation of cancer cells as compared with other laboratory animals or with man, was the subject of a series of investigations by the writer (13). Experiments with inoculations of normal tissue, with injections of aleuronat, scharlach R-oil, etc., have shown that the white rat reacts with a profuse connective tissue cell proliferation to all the different stimuli. All these facts and also

the remarkable phenomenon observed by Apolant (14), L. Loeb (15), and Bashford, Murray, and Haaland (16), that the new stroma found in the host under the influence of an implanted carcinoma may itself change into a sarcoma and subsequently suppress the carcinoma, indicate the importance of the stroma of cancer.

Conditions may yet be found under which a malignant transplantable tumor will be produced artificially in white mice or rats, and work with this end in view is in progress by the writer.

Nevertheless there will not be offered sufficient proof to the existence of a correlation between the reactive stroma formation and the transplantability of the cancers of the white rat or mouse, unless it be shown that a preliminary connective tissue stroma formation presents the only method whereby a tumor may be successfully inoculated. The following observation drew the attention of the writer forcibly to this question. The tumor which served for this investigation, a spindle-celled sarcoma of the white rat, grows, upon subcutaneous inoculation, in a manner similar to the one described by Russell for mouse carcinoma. There forms through the proliferation of the connective tissue cells of the host an extensive reactive stroma around the graft. This stroma is well vascularized, but in view of the sarcomatous structure of the inoculated tumor, the newly formed connective tissue does not penetrate to any appreciable extent between the parenchymatous cells of the tumor. Figure 1 shows very clearly this new stroma formation around a tumor graft three days after a subcutaneous inoculation. In its further development the tumor retains this surrounding stroma, does not infiltrate the musculature, fascia, or skin, and always remains a strictly encapsulated growth. This tumor, while very malignant, rarely forms metastases, and then usually in the lungs. In one animal, numerous metastatic nodules were observed in the liver. These metastases were not surrounded with a capsule, but showed a diffuse infiltrative growth in the parenchyma of the liver (figure 2). Thus the same tumor shows great differences in its manner of growth, when appearing in different tissues of the same organism.

The question then presented itself, whether different results in regard to reactive stroma formation could be obtained if the inoculation of the tumor were made into the parenchymatous organs

instead of subcutaneously. The present investigation was undertaken to answer this question.

Notwithstanding the great amount of research done during the last decade on transplantable cancers of the white rat and mouse, there have been very few attempts at inoculation of tumors into parenchymatous organs. Ehrlich (17) simply mentions the fact that Goldman successfully inoculated tumors for him into different organs. Similar successful inoculations are reported by Kraus, Ranzi, and Ehrlich (18), and by Graf (19), but none of these investigators seem to have paid any attention to the character of the stroma. Flexner and Jobling (20) twice succeeded in causing a rat tumor to grow in the testicle. They say that in the periphery of the tumor nodules multiplying tumor cells were lying in a fibrous stroma. Stumpf (21) inoculated mouse carcinoma into the kidney, and noted a certain diminution in the reactive stroma formation. His experiments did not seem to be very successful; he reported that in 80 per cent. of the operations the tumor fell out of the kidney and grew in the peritoneal cavity, and he stated also that it was very difficult to evade sepsis after the operation.

As was stated above, the present investigation was made with a transplantable sarcoma of a white rat, and the tumor was inoculated into the testicle, brain, and kidney. The following is a description of the experiments.

INOCULATION INTO THE TESTICLE.

This experiment was repeated on thirty animals. At first the operation was done with surgical technique, *i. e.*, a small incision was made in the tunica albuginea, a piece of the tumor was placed in the centre of the testicle, and the opening in the capsule was closed with a suture. In subsequent experiments a piece of the tumor was placed in the centre of the testicle simply by the aid of a trocar needle. The testicles were removed and examined at periods varying from twenty-four hours to four weeks after the inoculation.

On gross inspection of the organs, one notices that the tumor grows centrifugally from the inoculated piece to the periphery of

the organ, gradually replacing the testicle tissue. The organ increases in size under the pressure of the growing tumor, which ultimately breaks through the capsule and grows outside of the organ. The percentage of cases where the tumor did not grow in the testicle corresponds closely with the percentage of unsuccessful takes on a subcutaneous inoculation of the same tumor.

But the most striking picture presents itself on the microscopical examination of this sarcoma. The sarcoma cells spread diffusely between the seminiferous tubules replacing the strands of connective tissue cells of the organ. There is no indication of any connective tissue reaction, small round cell infiltration around the growing tumor, or any stroma formation. The tumor cells do not grow in one compact mass, but infiltrate diffusely and branch out into the different spaces between the tubules. Figure 3 illustrates the appearance of the tumor three days after the inoculation. The proliferation of the tumor cells is apparently just as active as it is three days after a subcutaneous inoculation (figure 1). But while in the latter case there is an extensive stroma formation around the group of proliferating tumor cells, there is no such connective tissue reaction in the testicle.

INOCULATION INTO THE BRAIN.

The operation consists in drilling an opening into the skull over the area of the right or left hemisphere. The hole is made large enough to permit the introduction of the trocar needle used for the inoculation. By the aid of this needle, a piece of the tumor is introduced into the brain, and then the skin incision is sutured.

The experiment was repeated on ten animals. One animal died from the operation and the rest were killed at periods ranging from twenty-four hours to ten days after the inoculation. On gross inspection one notices again, as in the testicle, that the tumor grows centrifugally, and replaces the brain tissue so as to occupy practically the greater part of a hemisphere. The microscopical picture is similar to the one observed in the testicle. The sarcoma cells infiltrate diffusely the brain substance. The margins of the growth show an extremely irregular form, and there is no indication of any

connective tissue stroma formation around the growth. Figure 4 shows the appearance of the tumor three days after inoculation into the brain and clearly illustrates all the points mentioned above.

All the animals developed symptoms of intracranial pressure. By placing the pieces of the tumor into different regions of the brain (which is technically easy of performance) it is possible that a great deal of information may be gained on the pathology of intracranial tumors. This subject will be considered in a future investigation.

INOCULATION INTO THE KIDNEY.

This operation is performed in the following way. The kidney is exposed by a lumbar incision and brought into the operative field by counter-pressure from in front. An incision is made with a fine scalpel along the convex border lengthwise of the kidney. A small piece of the tumor is inserted with forceps, the slit in the kidney closed with one suture of very fine silk, and the incision in the skin closed with a continuous suture.

The experiment was repeated on twenty-five animals. The percentage of cases, where the tumor did not grow corresponded with the percentage of unsuccessful takes on subcutaneous inoculation. In no instance did the implanted piece of tumor fall out of the kidney, nor did a single animal die as a result of the operation. The gross appearance of the tumor growth in the kidney is similar to the one observed in the testicle and brain. Microscopically one notices again complete lack of reactive stroma formation around the growing tumor. The sarcoma cells infiltrate the spaces between the tubules or glomeruli. When the tumor grows to a larger size, groups of glomeruli may be observed surrounded everywhere with sarcoma cells. Figure 5 illustrates the diffuse spreading of the tumor cells in the kidney and absence of connective tissue reaction around the growing edges of the tumor.

In attempting to analyze the significance of the results obtained by this investigation, one must bear in mind the fact that the connective tissue groundwork in tumors consists of two distinct parts. One is the specific connective tissue stroma between groups of cancer cells. This stroma, as was stated above, retains its structural

characteristics for each type of tumor, and its formation is probably due to a specific chemiotactic influence of the cancer cell upon the adjacent fibrous connective tissue or endothelium of the blood-vessels. In an artificially inoculated tumor, the formation of this specific stroma begins only after the implanted cancer cells become ingrafted and are enabled to use the nourishment of the new host. Only then do the cancer cells develop their power for unlimited proliferation and their chemiotatic influence upon the neighboring connective tissue cells. This latter part of the stroma plays a very subordinate rôle in the formation of a sarcoma, but it is demonstrable. Figures 7 and 8 show that this intercellular connective tissue stroma appears to be identical whether the sarcoma was inoculated subcutaneously or into the testicle. The other part of the connective tissue groundwork presents a layer of fibrous tissue, frequently accompanied by a small round cell infiltration, which surrounds and encapsulates the whole tumor and walls it off from the surrounding normal tissue. The presence of this surrounding stroma is not a general characteristic in human malignant tumors, and while it is very extensive in scirrhous, for instance, it may not be present at all in medullary carcinoma. Nor is the significance of the presence of this part of the stroma as uniform as the intercellular part, which latter in all cases apparently serves both for the support and nourishment of the cancer cells. In certain conditions, the formation of the surrounding stroma is a result of an inflammatory process which precedes the development or further growth of the tumor. This "precarcinomatous state of the surrounding connective tissue," as Bonney calls it, may facilitate the further growth, or even give rise to the formation of the tumor.

On the other hand, Borst (22) and Orth (23) have shown, that in certain tumors a connective tissue stroma may surround a group of cancer cells, gradually increase in size, compress the cancer cells and produce, as it were, a local cure of the process. In this case, Orth thinks that the primary factor is a degenerative change in the cancer cells, while the connective tissue stroma which forms subsequently is of the same nature and origin as the connective tissue capsule which forms around a foreign body or a conglomeration of

dead cells. The same explanation must be given apparently to the formation of the connective tissue capsule which was described by Schmidt (24) as taking place around a tumor embolus which penetrates into the lumen of the blood vessels.

In transplantable tumors, provided the inoculation is subcutaneous, the success of grafting depends upon the formation of a connective tissue stroma around the implanted piece. By the subcutaneous method, the piece of tumor is placed in a loose pocket at a great distance from the blood vessels either of the skin or the muscles. Unless the inoculated piece is surrounded promptly by a round cell infiltration and by fibroblasts, and unless a connective tissue and vascular stroma is formed, the tumor cells can not become ingrafted, and die from starvation. But the formation of this primary surrounding stroma is not due to any *specific* influence of the cells of the graft. As was stated above, this specific chemiotactic influence of the implanted cancer cells develops only subsequently, and then serves to form the specific intercellular stroma. A piece of normal tissue or any foreign body is surrounded by a similar vascular connective tissue stroma when introduced subcutaneously into a rat. Whatever the cause of the formation of primary stroma after a subcutaneous inoculation, the influence upon the cells of the host is identical, whether a piece of normal tissue or a piece of a malignant tumor be used for inoculation.

Levin and Sittenfield (25) have shown, in a recent communication, that the success of a tumor implantation depends upon two factors: first, whether the implanted piece becomes ingrafted, and second, on the capacity of the implanted cells to proliferate indefinitely in the new host. The formation of the surrounding stroma is of importance only for the success of the first step, the ingrafting, and it has no influence upon the subsequent proliferation of the inoculated cells and the formation of a malignant tumor. Figure 6 shows extensive connective tissue stroma formation around a piece of a mouse sarcoma inoculated subcutaneously into a rat. The cells of this mouse tumor obtain at least as good a primary surrounding stroma as they would in a mouse, and they even proliferate for a few days, but ultimately they become absorbed and do not form a malignant tumor in the foreign host.

But what is of much more importance, the present investigation shows conclusively that the formation of a primary surrounding stroma is not a necessary condition for the success of the ingrafting. When a piece of tumor is inoculated into a parenchymatous organ, it is placed so near the blood-vessels of the organ that it is enabled to obtain immediately the necessary nutrition. As a result, the first visible step after such an inoculation is not the formation of new connective tissue, but the proliferation of the cancer cells themselves. The specific intercellular stroma is formed later, after the implanted cancer cells become adjusted to the conditions of nutrition in the new host.

The present investigation tends to show further, that the absence of "the specific stroma reaction" in immune animals can not have a general application in the explanation of the phenomenon of the resistance of certain animals against the inoculation. It may serve only to explain the success or failure of the grafting of a tumor subcutaneously. Not only does this theory fail to explain the further growth and development of the inoculated tumor, but it is also inadequate to explain the first stage of grafting when the inoculation is made into a parenchymatous organ, since a stroma formation does not represent the first step of such an inoculation.

The study of the fate of tumor tissue when inoculated into the parenchymatous organs of immune animals will be the subject of further research.

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EXPLANATION OF PLATES.

Plate LXXX.

Fig. 1. A graft of sarcoma three days after subcutaneous inoculation. The right side of the figure shows the part of the graft which became necrotic, and the centre, the proliferating part of the tumor which is surrounded to the left, above and below, with connective tissue stroma.

Fig. 2. The centre shows a metastasis of the sarcoma infiltrating diffusely into and surrounded by normal liver tissue.

Plate LXXXI.

Fig. 3. A sarcoma graft in a testicle, three days after introduction. The centre of the figure shows the sarcoma cells infiltrating diffusely between the seminiferous tubules.

Fig. 4. A sarcoma graft in a hemisphere of the brain, three days after introduction. The centre of the figure shows the sarcoma cells diffusely infiltrating the brain substance. There is no indication of formation of stroma.

Plate LXXXII.

Fig. 5. A sarcoma graft in a kidney, three days after introduction. The right side of the figure shows the necrotic part of the graft, and the centre, the proliferating part of the tumor, which invades diffusely the kidney tissue to the left. There is no indication of stroma formation.

Fig. 6. A graft of mouse sarcoma in a rat, three days after subcutaneous inoculation. The centre of the figure shows a small group of sarcoma cells surrounded everywhere by a connective tissue stroma. Numerous giant cells are seen.

Plate LXXXIII.

Fig. 7. A sarcoma graft in a kidney, three days after inoculation. It shows a few fibrillæ between the sarcoma cells and around the kidney tubules. No con-

FIG. 1.

FIG. 2.

Mali

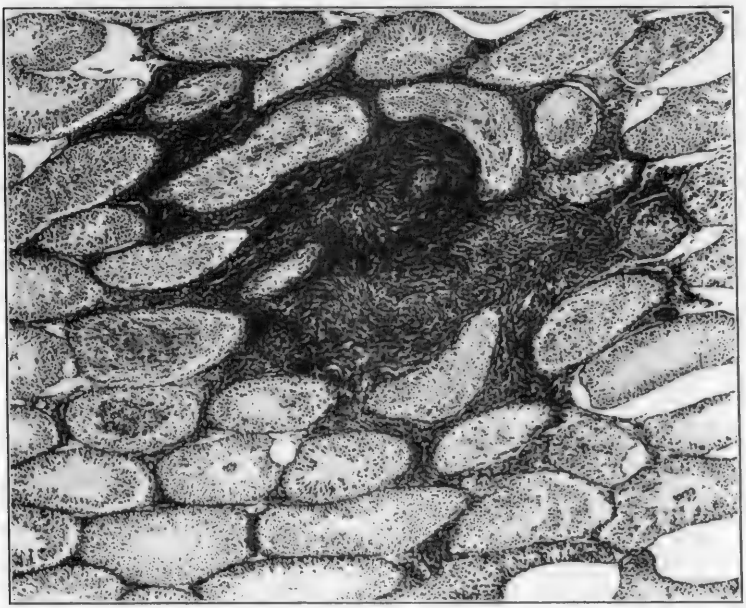

FIG. 3.

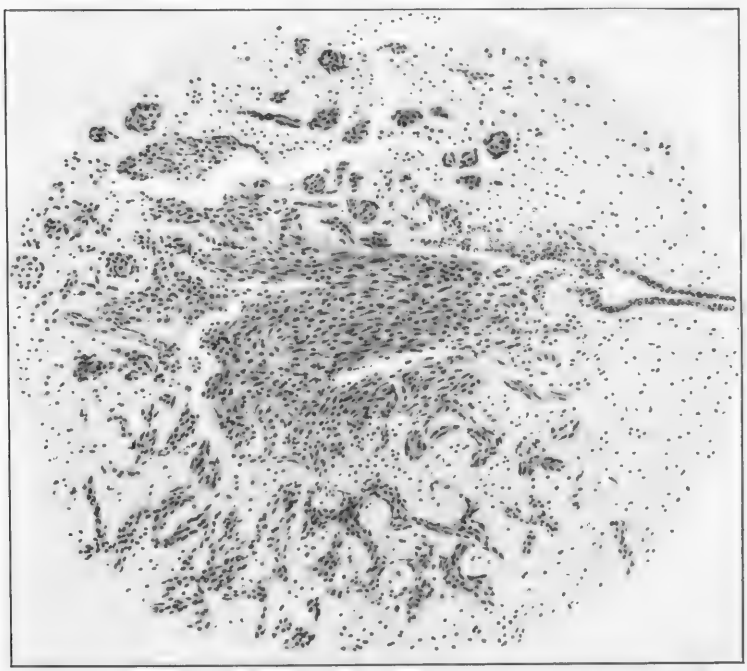

FIG. 4.

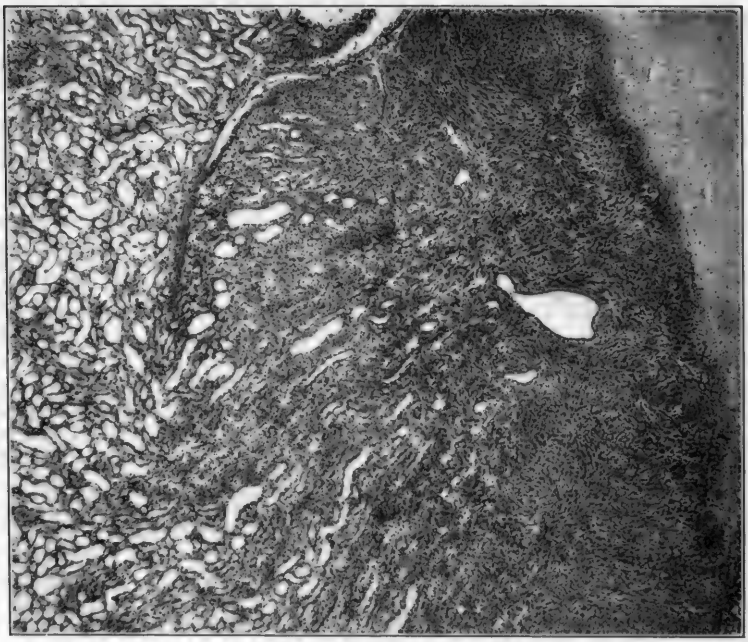

FIG. 5.

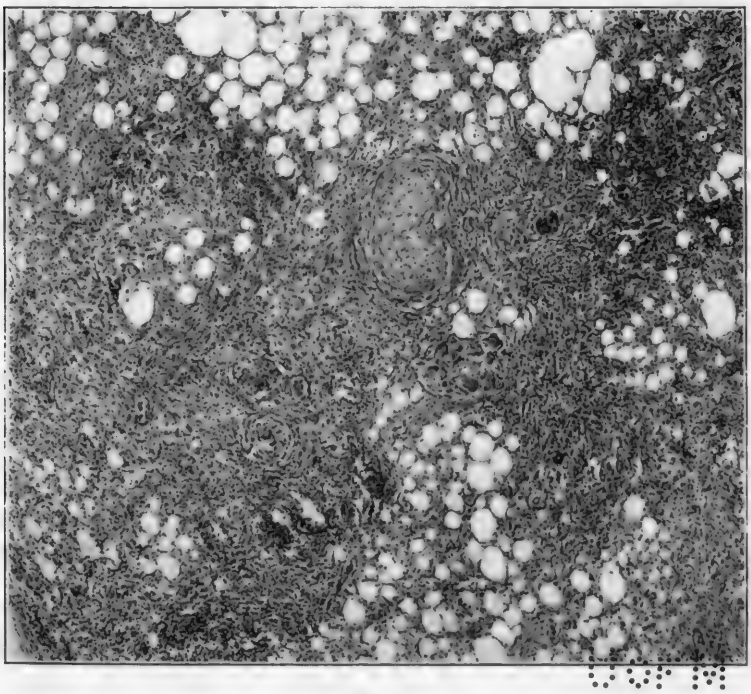

FIG. 6.

FIG. 7.

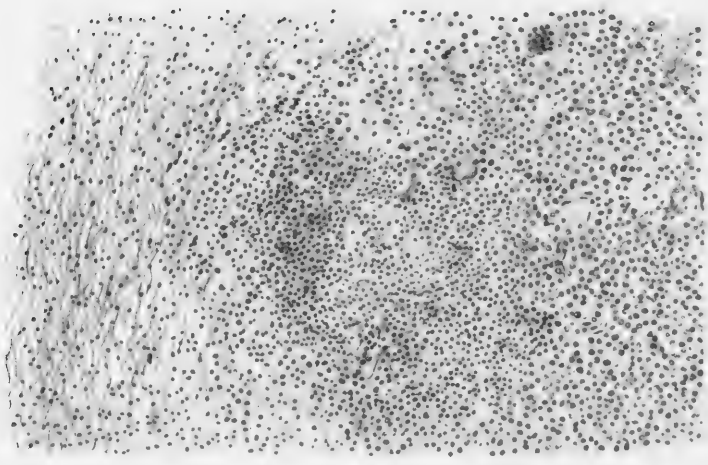

FIG. 8.

Univ of M

Mau

nective tissue rich in fibrillæ divides the tumor from the normal kidney. Stained with Mallory's anilin-blue.

FIG. 8. A graft of sarcoma, three days after its subcutaneous introduction. The right side of the figure shows sarcoma cells which are surrounded to the left by a small round cell infiltration and still further to the left by a connective stroma formation. The latter contains an abundance of fibrillæ. A few intercellular fibrillæ are seen between the sarcoma cells. Stained with Mallory's anilin-blue.

THE MICROGRAPH.

An Instrument Which Records the Microscopic Movements of a Diaphragm by Means of Light Interference, and Some Records of Physiological Events Showing the Registration of Sound Waves Including the Human Voice.*

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Plates LXXXIV–XCII.

A new instrument for observing and recording small changes of mechanical pressure by making use of the phenomena of the interference of light has recently been described.[1] Several important changes in the instrument have been made since the original communication, and the development of its applications has been in steady progress. In the present paper, an account is given of some results obtained on normal subjects in the effort to ascertain the characteristics and possibilities of the instrument. At certain portions of the cardiac cycle, changes take place with such rapidity that it requires an instrument free from any considerable inertia in the moving parts to register them accurately.

In instruments heretofore in use, the mass and inertia of the moving parts and the distance through which they are required to move have been considerable. At the points of most rapid pressure-changes in the cardiac cycle, such instruments overshoot the mark on the up stroke and then again on the return because of the inertia. The important feature of the new instrument is that it reproduces

* Received for publication, April 5, 1911.

[1] George O. Squier and Albert C. Crehore, Note on Oscillatory Interference Bands and Some Practical Applications, *Bulletin of the Bureau of Standards*, 1911, vii, 1.

faithfully all pressure changes without the disturbing effect of inertia. This is due to two causes: first, the mass of the moving part is small and is controlled by the strong elastic force of the diaphragm, and second, the motion required of it is microscopic; in fact it can not be seen to move at all with the unaided eye. By means of the interference of light, however, the motion of the diaphragm itself is magnified about 50,000 times, a magnification so great that the motion of the diaphragm becomes directly visible to the eye.

THE MICROGRAPH.

The instrument contains a thin metal diaphragm similar to that of a telephone, on one side of which there is an air chamber connected by a tube to a tambour receiver located on any desired portion of the body. There is thus a confined volume of air, one portion of which bears upon the body, which acts as a transmitter, and another portion upon the telephone diaphragm which acts as a receiver. This diaphragm is free to respond to all the vibrations transmitted to it through the medium of the confined air column, and the motion produced in the diaphragm is too small to be observed directly by the unaided eye. It is for the purpose of observing the motion of this diaphragm that the phenomena of the interference of light are used, as has been said, and this enables us not only to observe the motion visually but to obtain a photographic record from which most accurate measurements may afterwards be made at leisure. The instrument is thus divided into two distinct parts, (1) that required for observing the motion of the diaphragm visually, and (2) the additional apparatus required when photographs are to be made.

A sectional view of the instrument proper is shown, reduced two-fifths, in figure 1. In addition to the source of light, this instrument, connected by tube attached at the outlet, *T*, to an ordinary tambour, is all that is required for observing the motion of the diaphragm. The air space, *C*, connected to the outlet, *T*, through a hole in the base, *A*, is confined on its upper side by the diaphragm, *D*, which is clamped by means of the ring, *B*, to the base. A change of pressure of the air in the chamber, *C*, causes a slight imperceptible deflection of the diaphragm, which for motions as small as a few wave-lengths of light is strictly proportional to the change of pressure of the air.

The rest of the instrument is the optical portion for the purpose of making visible these small movements of the diaphragm. The only really satisfactory

method of magnifying these almost infinitesimal movements is by the use of light interference. A plane mirror, *E*, polished on its upper surface so as to make a metal reflector is supported by means of a small block, *F*, at the center of the diaphragm and forms a part of the diaphragm, participating in all of its movements. Just above this reflector is supported a cylindrical plano-convex lens, *L*, with the plane surface on the under side so that it may be adjusted parallel with and close to the reflector, *E*, without touching it. This adjustment is accomplished by means of the three screws, *S*, threaded in the brass ring, *G*, to which the cylindrical lens is attached, and resting upon the brass base, *A*. Three spiral springs hold the ring, *G*, to the base, *A*, and produce a uniform pressure at all times upon the screws, *S*, which form the only points of contact between the upper portion and the base of the instrument.

If a mercury vapor electric lamp is placed directly above the instrument and an observer looks down into it, a series of brilliant interference bands of light is seen between the two reflecting surfaces, the lower, *E*, being attached to the diaphragm and the upper being the plane surface of the cylindrical lens attached to the frame. The position of these interference bands depends upon the adjustment of the lens, that is, upon the distance apart of the two surfaces and whether they are parallel. When the surfaces are separated by a small distance and are parallel the bands appear circular in form, consisting of a series of concentric rings.

As the diaphragm moves, the reflecting surface, *E*, approaches and recedes from the lens, and this motion causes the circular bands to contract and expand respectively, but they remain of a circular form and the center of the system does not move. As they contract with an increase of pressure in the air chamber, the central bands disappear one after another while the pressure increases, and when the pressure decreases they reappear again successively at the center and expand. The movements of these bands may be seen by an observer looking directly into the instrument, but to obtain all the details the motion may be recorded upon a moving photographic plate or film arranged to move transversely across a slit which admits the light to the plate. The bands form a real image upon the ground glass of the camera and appear as shown in figure 2. The slit through which light is admitted to the plate is shown at *AB* and the direction of the motion of the plate is indicated by the arrow. When the circular bands expand and contract simultaneously with the movement of the plate past the slit, each band leaves its trace

upon the film in the form of a wavy line. At the center of the circular system of bands, P_1, P_2, P_3, P_4, etc. represent points where the central bands contract to zero and disappear, and reappear on expansion leaving a record upon the plate. The number of such points represents the number of bands displaced successively.

When one interference band expands or contracts so far that it takes the exact place of the next adjacent band, then it is known that the two reflecting surfaces have receded or approached by a distance exactly equal to one-half of one wave-length of the particular kind of light employed. Since the wave-length of light is a definite well-known distance, the exact distance through which the diaphragm moves in a definite time becomes accurately known by counting the number of bands which appear and disappear in any given distance along the plate. It follows that an accurate curve representing the pressure changes of the air in the chamber of the instrument with the time, that is, a so-called "pressure-time curve," may easily be constructed because, as has been stated, the deflections of the diaphragm are strictly proportional to the pressure of the air for small deflections.

TABLE I.

Table of Measurements on Negative. "I. B.," Dec. 8, 1910. 6.3 inches per second. See curve, figure 4.

Band.	Inches.		Band.	Inches.	
9	0.08		2	4.32	
10	0.22		1	4.52	Minimum
	0.52	Maximum	2	4.78	
10	0.79		3	5.29	
9	1.05		4	5.58	
8	1.24		5	5.95	
7.2	1.35	Level	6	6.50	
7.2	2.00		7	6.80	
7	2.20		7.5	7.00	Level
6	2.51		7.5	7.31	
5	2.89		8	7.52	Maximum
4	3.34		7.5	7.76	Minimum
	3.60	Sharp fall	8	7.87	
3	3.70		9	7.96	
2	3.82		10	8.05	
1	3.94	Minimum	11	8.20	
2	4.05		12	8.65	Maximum
2.3	4.22	Maximum	11	9.15	
			10	9.62	

The manner in which a curve may be drawn from the photo-

graph is shown in detail in figures 3 and 4. Figure 3 is the photographic record and figure 4 the pressure-time curve obtained from it. The table gives measurements made on the negative from which the curve is constructed. In the first column appears the number of the band, and in the second, the distance in inches measured along the plate from an arbitrarily selected origin to the point where the band of that number appears or disappears. Since the photographic film is moved by a motor at a uniform speed which is known, these distances in the table may be converted into time by multiplying each by a constant factor. In plotting a curve, any band may be selected as a starting point and designated by any desired number because we are concerned not with the absolute values of the pressure but with relative values only; but after determining the number of a certain band, the band adjacent on one side takes a number one unit greater, and on the other side one unit less, all the bands being numbered consecutively from one end of the record to the other. The same band frequently passes by several times in the course of a record and it always has the same number associated with it.

With some experience, it is not necessary to make such a table of measurements to construct a curve. The distances are easily transferred with a pair of dividers directly from the negative to the chart, thus saving time and labor by avoiding the use of any scale. With more experience, the construction of a curve becomes unnecessary, as it is not difficult to recognize its form by an inspection of the negative. The distances or times of any critical points, such as maxima and minima, and points of most rapid change may be seen at a glance. Moreover, the trace of the bands furthest from the center shows the form of these curves on the negative itself, and in many cases this suffices, although the displacements are not exactly proportional to the pressures. Figure 5 is a photographic record having the center of the circular system of bands located near the edge of the record instead of at the center, and the curves seen below the center, by following one of the bands continuously from one end of the record to the other, conform in general features with a curve drawn from this negative by the above method. That is, the two curves agree throughout in their direction of motion, but the curve on the negative does not represent true amplitudes.

THE MAGNIFYING POWER.

The power of the method of light interference in magnifying the true motion of the diaphragm of the instrument may easily be calculated as follows: The wave-length of light used for photography employing the mercury vapor lamp is 40.47×10^{-6} centimeters, and if the diaphragm of the instrument moves one half a wave-length, the interference bands move exactly one whole band. The size of the image formed by these bands at the distance of the film is such that the diameter of the first band is approximately one centimeter. The diameter of this band, therefore, changes one centimeter while the diaphragm moves through a distance of 20.23×10^{-6} centimeters, and the magnifying power is the ratio of 1 to $20.23 \times 10^{-6} = 49{,}400$. This figure may be increased or decreased somewhat by enlarging or diminishing the image of the bands, but there is no great advantage in this on account of the indefinite character of the edges of the bands.

When compared with the best microscopes, it is seen that the magnifying power of the interference method is twenty to twenty-five times greater.

WAVES OF SOUND RECORDED.

As the speed of the film is increased, the time of exposure of a given point of the negative is reduced. Knowing the width of the slit and the speed of the plate, the time of exposure may be calculated. For example, in securing the records accompanying this paper the width of the slit was a fixed distance, about 0.05 inch. The highest speed of plate employed was fifty-eight inches per second. Hence, a given point on the plate passed the slit in $0.05/58 = 0.00086$ second, and the time of exposure was therefore less than the one thousandth part of a second.

To secure any record at these speeds requires an intense light. The question of sufficient light intensity for these rapid records is one of the difficulties which has been largely overcome. Since a nearly monochromatic source of light is required, the common carbon arc lamp can not be used, but the use of the mercury vapor arc and the employment of a single plano-convex cylindrical lens has made it possible to work at the speeds mentioned, although the higher

the speed the less dense the negative. Figure 19 gives the tracings from two negatives, the records being produced by singing the vowel "Ō" for the one and "Ē" for the other on the key of "G" in the second octave below middle "C." Certain of the higher harmonics in the case of the letter "O" are too rapid to be reproduced.

One of the features of the higher speed cardiograms is the recording of sound waves which are ordinarily heard with a stethoscope. Some of these are shown on the tracings in figures 9, 10, and 16.

PRACTICAL APPLICATIONS OF THE MICROGRAPH.

EXPLANATION OF PLATES LXXXIV–XCII.

PLATE LXXXIV.

THE INSTRUMENT.

FIG. 1. The instrument. A = the base; B = a ring clamping the diaphragm, D, to the base, enclosing an air-chamber, C, connected at the center with an outlet, T. A reflector, E, is supported on a block, F, upon the center of the diaphragm, D. The ring, G, carries a plano-convex cylindrical lens, L, with its plane surface nearest the reflector, E, and is adjustable by the three screws, S, which are threaded in the ring, G, and bear upon the base, A, passing through clearance holes in the ring, B. Three spiral springs around the circumference hold the ring to the base.

Interference bands are formed between the under surface of the lens, L, and the upper surface of the reflector, E, when the surfaces are adjusted parallel.

FIG. 2. The left of the figure represents an image of the band system as formed on the ground glass of the camera, and AB shows the position of the slit in the camera.

The right of the figure represents the paths of the moving bands as traced on the film passing the slit AB.

PLATE LXXXV.

ILLUSTRATIVE CURVE AND PHOTOGRAPHS.

FIG. 3. Photograph of moving bands from which the table of measurements (table 1) was derived and the curve in figure 4 plotted. The center of the bands is in the center of the plate.

FIG. 4 illustrates the curve plotted from the photograph in figure 3.

FIG. 5. Photograph of moving bands from which the lower curve in figure 6 is plotted. The center of the bands is on the edge of the plate. Six successive cardiac cycles are recorded.

At one portion of each cycle, corresponding to the dotted line in figure 6, the motion of the bands is too rapid to be recorded at this speed of plate.

Plate LXXXVI.

THE APEX BEAT.

Fig. 6 shows, in the lower portion, a curve of an apex beat plotted from a plate moving at the rate of 2.1 inches per second, the heart beating at 86 cycles to the minute, or about 0.7 second per cycle.

The upper portion shows the curve of an apex beat taken from a plate moving at a speed of 5.96 inches per second, the heart beating at 81 cycles to the minute or 0.73 second per cycle.

Each of these curves shows two features in common; first, three waves of increased pressure on the upstroke, and second, an interrupted line on the downstroke, designating that at this point on each curve the bands moved too rapidly to be registered separately at these rates of speed.

Fig. 7 shows two more curves of apex beats taken from plates moving at the rate of 10.85 inches per second. They show the same characteristics as those in figure 6, but at this speed they begin to bring out more detail.

Plate LXXXVII.

THE APEX BEAT.

Fig. 8 shows three more apex curves, each at a rate of 10.85 inches per second as in figure 7, and each bringing out the characteristic waves, but the contour of the curve as a whole varies, as do those of the lever cardiogram, owing probably to the varying site of the tambour with reference to the exact apex impulse.

Fig. 9 is recorded from two plates each moving at a rate of 22.25 inches per second, and shows the detail of the upstroke and its three major waves. It will be seen that superimposed upon these three major waves are minute waves, or rather that the major waves are constituted throughout of minor waves representing vibrations varying in these figures from 166 to 333 per second. In the upper curve the wave-lengths only are indicated, while in the lower, the complete curve including the amplitude is drawn. The number of complete vibrations per second is indicated by "n."

Plate LXXXVIII.

THE APEX BEAT.

Fig. 10, at the same speed, continues the detail of the curve of which figure 9 is a part. It covers that portion of the curve in figure 6 represented as a dotted line, in which the bands were moving too rapidly to be registered separately on a plate moving at the slower speed. Here again the letter "n" represents the numbers of complete vibrations per second.

While it may be objected that these vibrations are extraneous to the heart, in the chest wall, or from the environment of the patient, we shall express our opinion, based on the regularity of their recurrence in curve after curve, that they represent vibrations having different characteristics in different parts of the cycle, originating perhaps in the contraction of the cardiac muscle, or in the valves, or in the blood current. The fact that the frequency varies rapidly in a small fraction of a second proves that these vibrations are not due to the instrument *per se*.

FIG. 11 shows the curves from figures 5 to 10 reduced to the same scale. This figure shows how persistent in all these curves are the fundamental characteristics, while the feature of each individual curve varies. This is in agreement with the results obtained by the lever.

PLATE LXXXIX.

THE RADIAL PULSE.

FIGS. 12 and 13 are plotted from plates recording the events of radial pulses, the familiar contour of which appears at once, and it seems all the more remarkable at first thought when it is considered that the movement of no lever was concerned in the production of this latter curve, which is only the plotted expression in time and pressure of what is going on in the pulse as recorded in the photograph of moving interference bands of light; but on second thought, it is appreciated that this curve is a true pressure-time curve, which the lever tracing inadequately approximates.

These curves of normal pulses all show the characteristic dicrotic notch, a sloping upstroke, and a rounded plateau.

PLATE XC.

THE JUGULAR VEIN.

To the student of cardiac disease, tracings of the jugular pulse are of dominant interest.

FIG. 14 shows four of these cycles taken from the jugular vein of a normal subject, the heart beating at 70 to the minute. The speed of the plate is 5.98 inches per second. The remarkable repetition of these tracings plotted from four different cycles on the negative attest the accuracy of the registration of the pressure changes within the vessel. To analyse these tracings in terms of the lower tracings is not so easy a task as in the case of the radial and apex curves. For this reason we felt it necessary to obtain tracings for comparison from the bared jugular vein of a dog (see figure 15).

FIG. 15 shows seven curves taken from the bared jugular vein of a dog. Compare the details with those of the human subject in figure 14. The striking similarity in the waves, allowing for difference in amplitudes is obvious. Curves 51 to 55 are taken from plates moving at the rate of 11.25 inches per second. Curves 56 and 57 are portions of a cycle from a plate moving at the rate of 23.3 inches per second, about twice as fast as the preceding, on which sound vibrations are determined ranging from 140 to 350 periods per second.

Note the rapid fall in the pitch of the sound, *i. e.*, from 350 to 181 complete cycles in about 0.02 second, as the summit of the wave is passed.

PLATE XCI.

THE JUGULAR VEIN.

FIG. 16 shows two more curves taken from the bared jugular of a dog, the plate moving at 10.9 inches per second, the heart beating at 160 and 170

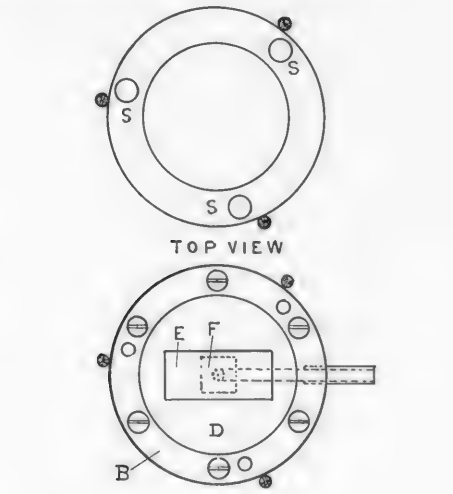
S
S
S
TOP VIEW
E
F
D
B
VIEW ON J K
S
S
L
F
E
G
B
J
K
A
C
D
T
SECTIONAL VIEW

FIG. 1.

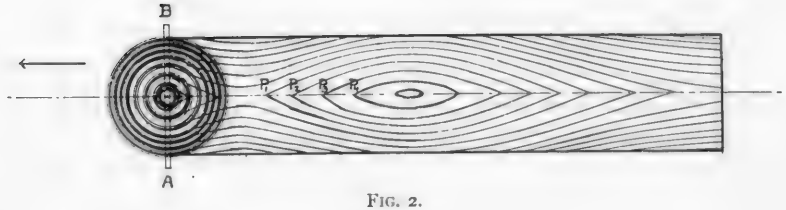
B
A

FIG. 2.

M'Gill

FIG. 3.

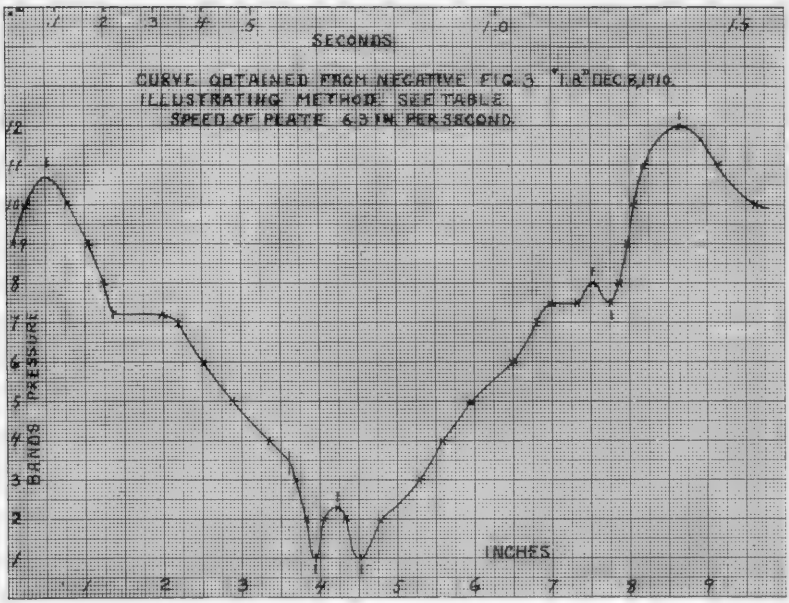
SECONDS
CURVE OBTAINED FROM NEGATIVE FIG. 3 "I.8" DEC 8, 1910.
ILLUSTRATING METHOD. SEE TABLE.
SPEED OF PLATE 6.3 IN. PER SECOND.
BANDS PRESSURE
INCHES

FIG. 4.

FIG. 5.

U of M

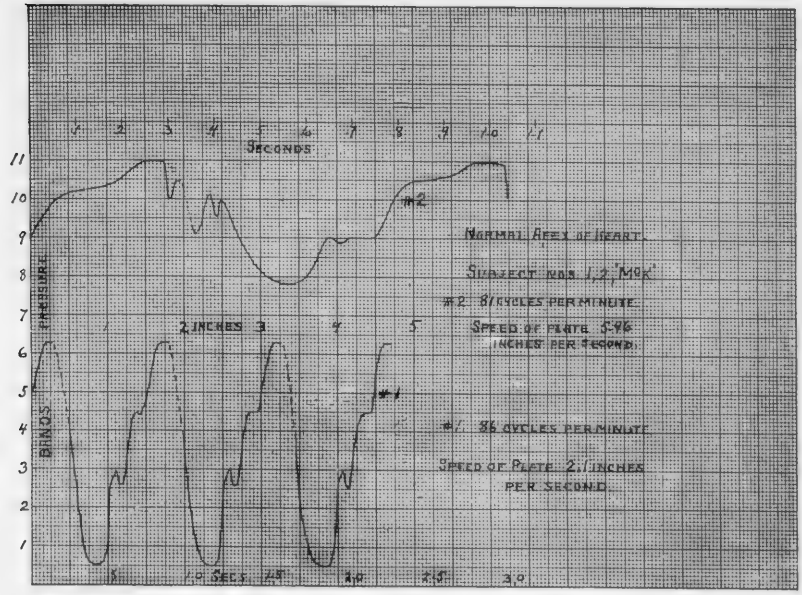
SECONDS
#2
NORMAL APEX OF HEART.
SUBJECT NOS. 1, 2, "M.K."
#2 8 CYCLES PER MINUTE.
SPEED OF PLATE 5.96 INCHES PER SECOND.
#1
#1 86 CYCLES PER MINUTE
SPEED OF PLATE 2.1 INCHES PER SECOND.
BARDS PRESSURE
INCHES
SECS

FIG. 6.

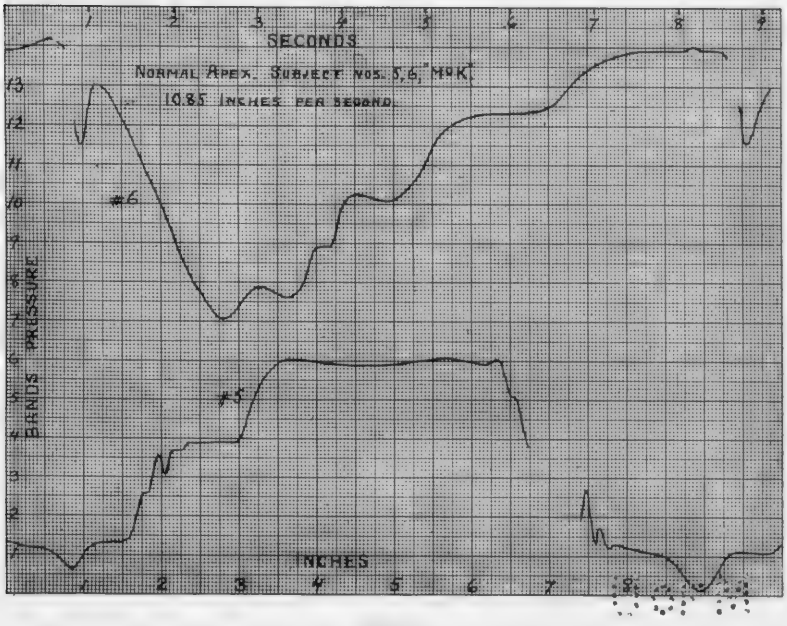
SECONDS
NORMAL APEX. SUBJECT NOS. 5, 6, "M.K."
10.85 INCHES PER SECOND.
#6
#5
BARDS PRESSURE
INCHES

FIG. 7.

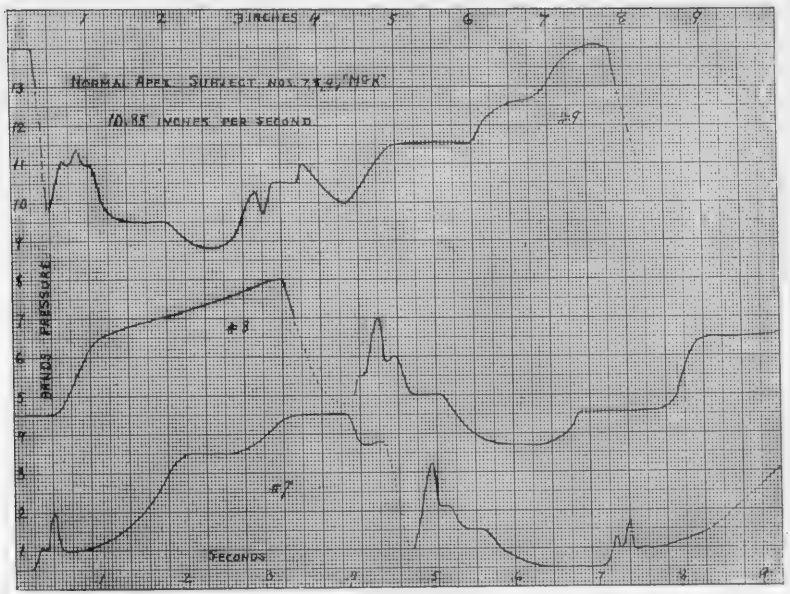

FIG. 8.

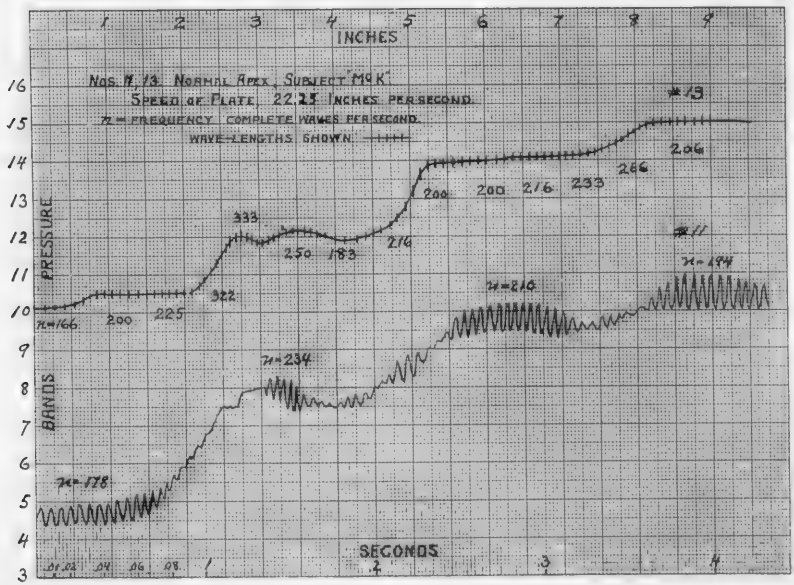

FIG. 9.

U of M

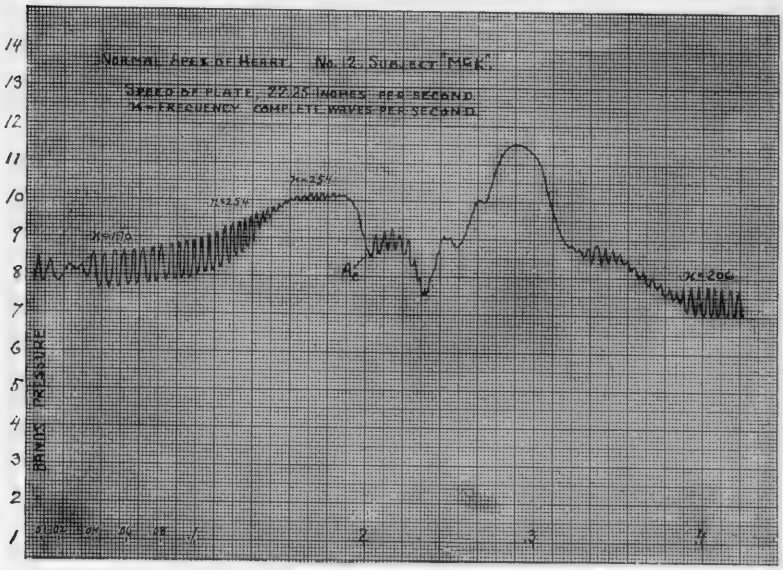

FIG. 10.

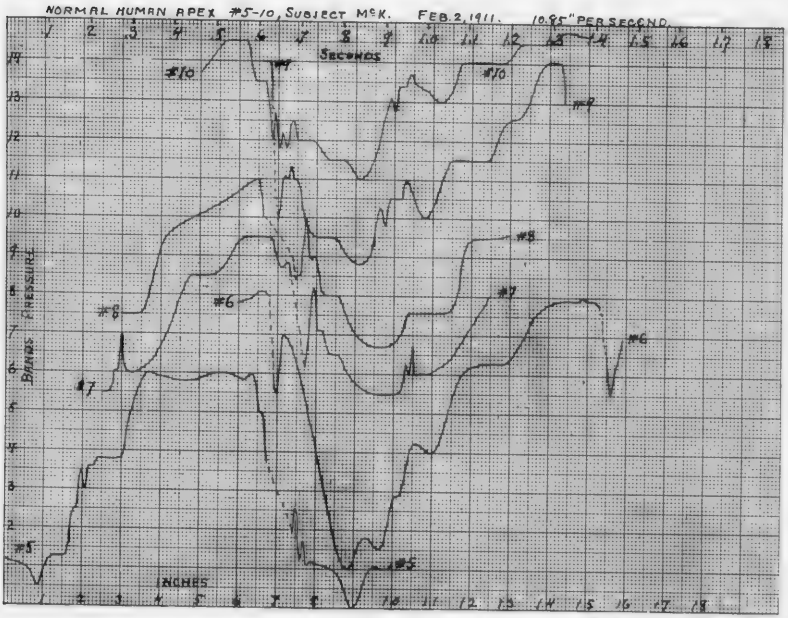

FIG. 11.

UofM

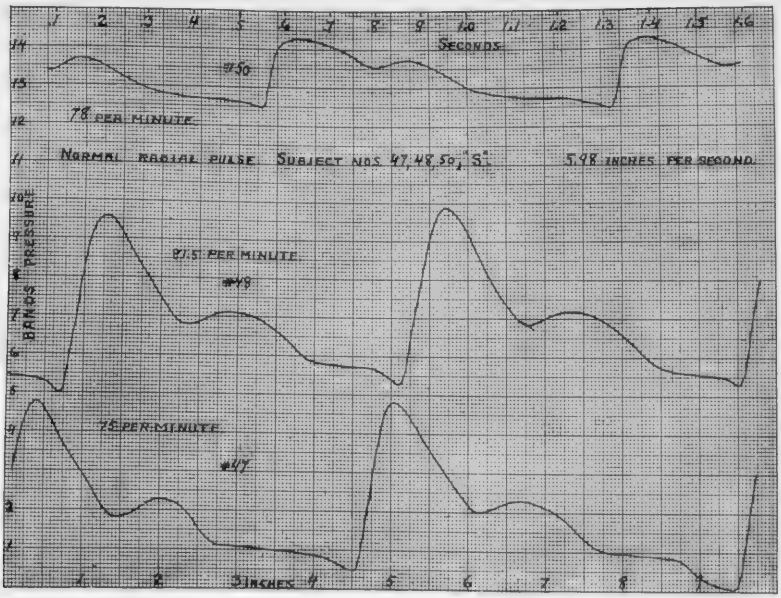
SECONDS
#50
78 PER MINUTE
NORMAL RADIAL PULSE SUBJECT NOS 47, 48, 50, S.
5.98 INCHES PER SECOND
BANDS PRESSURE
#48
75 PER MINUTE
#47
75 PER MINUTE
INCHES

FIG. 12.

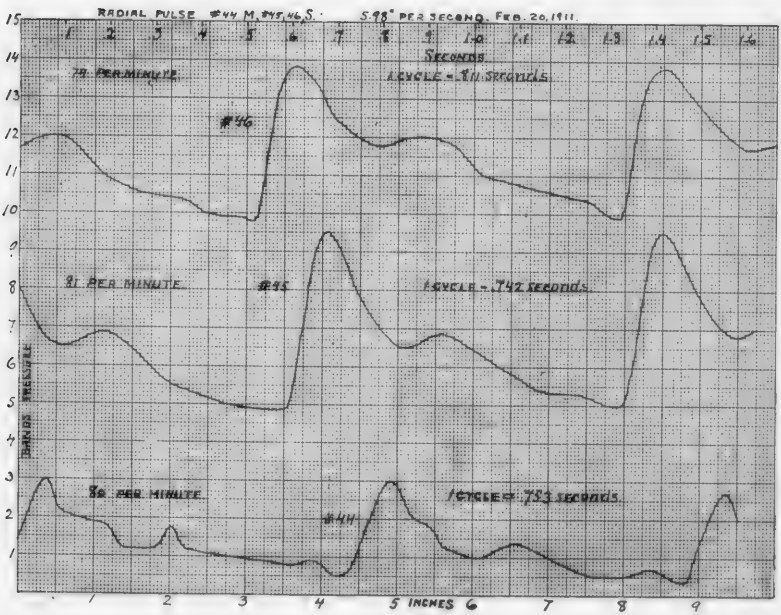
RADIAL PULSE #44 M. #45, 46, S. 5.98" PER SECOND. FEB. 20, 1911.
SECONDS
78 PER MINUTE
1 CYCLE = .811 SECONDS
#46
81 PER MINUTE
#45
1 CYCLE = .742 SECONDS
BANDS PRESSURE
80 PER MINUTE
#44
1 CYCLE = .753 SECONDS
5 INCHES

FIG. 13.

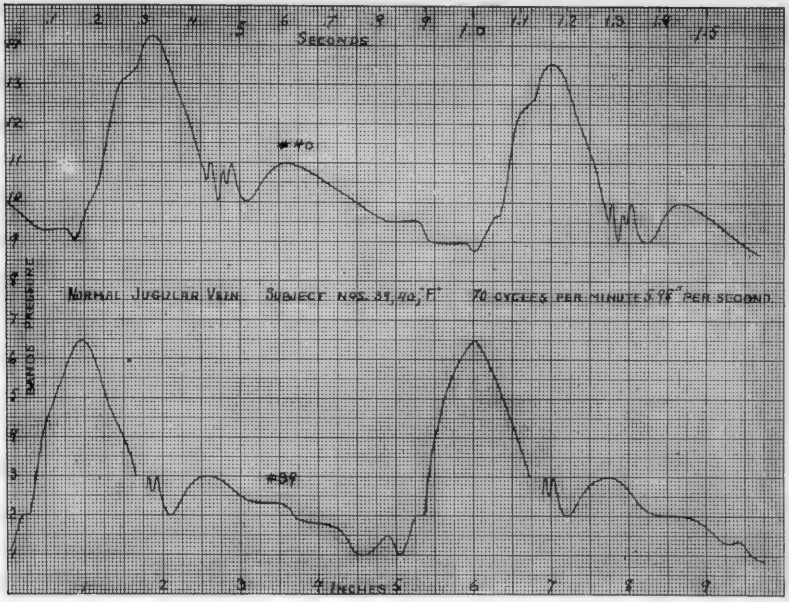
SECONDS
#40
NORMAL JUGULAR VEIN SUBJECT NOS. 31, 40, F. 70 CYCLES PER MINUTE 5.75" PER SECOND
#39
INCHES

FIG. 14.

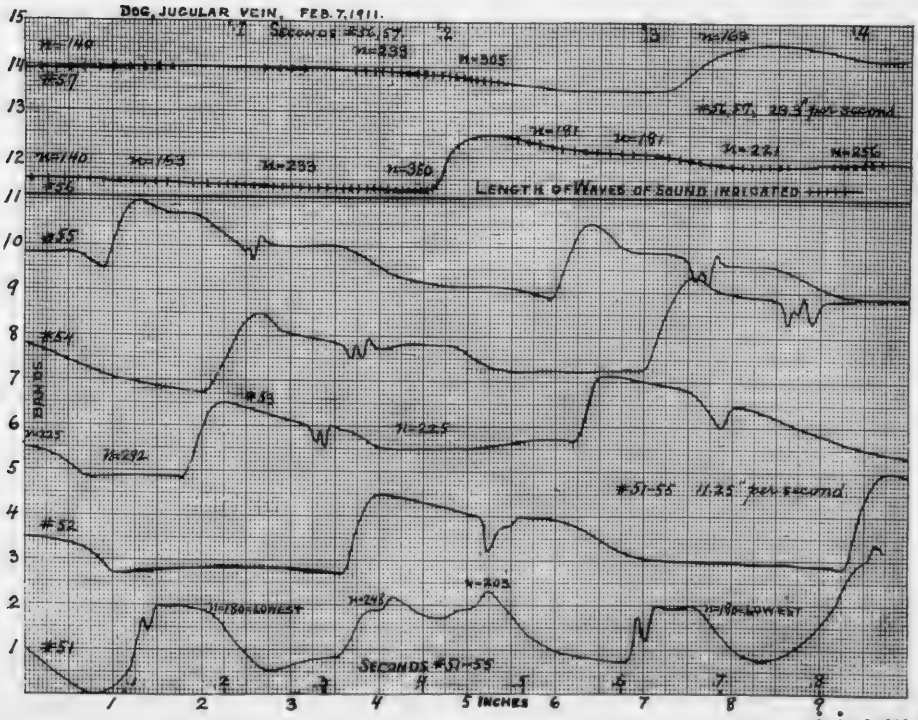
DOG, JUGULAR VEIN. FEB. 7, 1911.
SECONDS #56, 57
#57
#56
#55
#54
#53
#52
#51
SECONDS #51-55
#51-55 11.25" PER SECOND
LENGTH OF WAVES OF SOUND INDICATED
INCHES

FIG. 15.

Mau

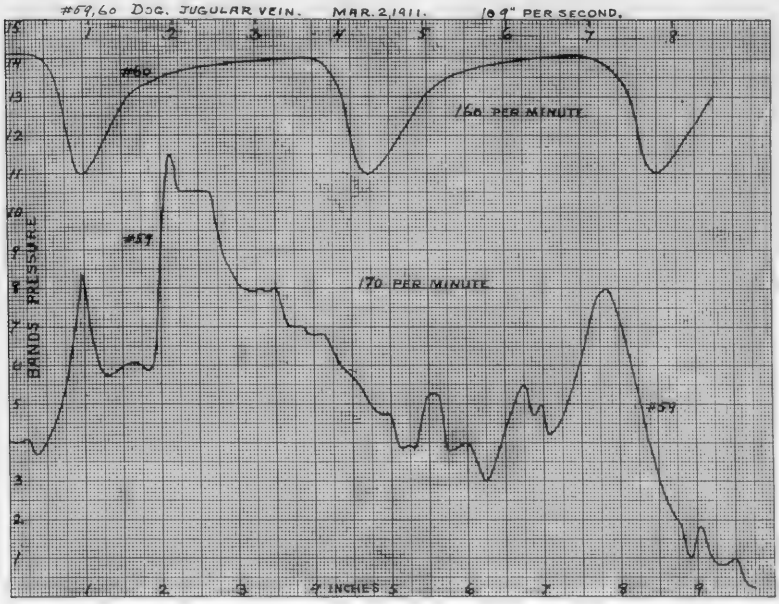

FIG. 16.

FIG. 17.

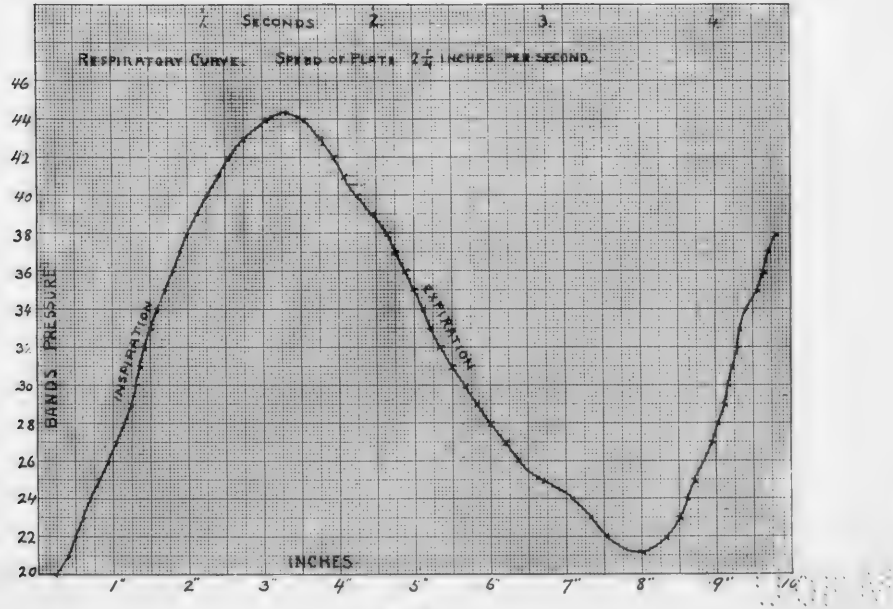

FIG. 18.

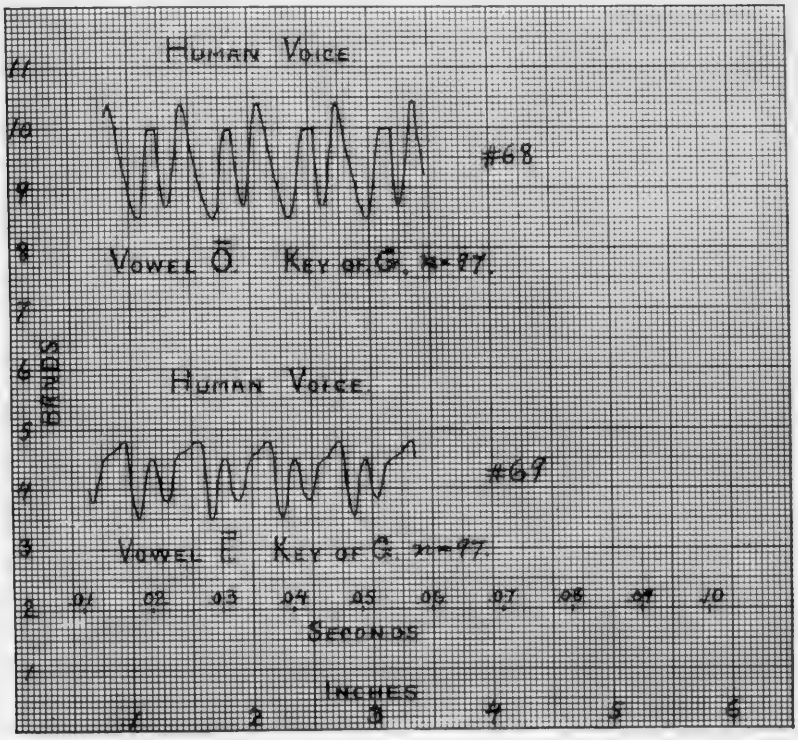
Human Voice
#68
Vowel Ō Key of G, N=97.
Human Voice
#69
Vowel Ē Key of G N=97.
Seconds
Inches

Fig. 19.

Fig. 20.

per minute. The lower curve shows with what accuracy rapid changes of pressure are recorded in hearts beating at a rate which in the human subject constitutes a tachycardia.

FIG. 17. Photograph of bands, showing sound waves, taken from the bared jugular vein of a dog.

RESPIRATORY CURVE.

FIG. 18 is a respiratory curve taken from a plate moving 2.25 inches per second. It suggests at once the use of the instrument in the study of respiratory movements, physiological and pathological.

PLATE XCII.

THE HUMAN VOICE.

FIG. 19 shows curves of the human voice plotted from the photograph in figure 20.

FIG. 20. Photograph of bands, showing sound waves, of the vowel "E," in the key of "G," sung into the instrument. "n" = 97.

AN EXPERIMENTAL STUDY OF THE QUESTION OF ASPIRATION OF FOREIGN MATERIAL INTO THE AIR PASSAGES DURING INTRATRACHEAL INSUFFLATION.*

BY T. S. GITHENS AND S. J. MELTZER.

(*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research, New York.*)

Two years ago Meltzer and Auer[1] published experiments showing that by insufflation of a continuous stream of air through a tube reaching to the lowest part of the trachea, the life of curarized animals could be sustained for four hours or longer. In these experiments the tube was introduced through an incision in the trachea; and the diameter of the tube had to bear a definite relation to that of the trachea. Further experimentation upon the subject showed that similar results could be attained even when the tube was introduced into the intact trachea through the mouth and larynx. In this case, however, the tube had to be of a smaller caliber than when introduced through an incision in the trachea. Furthermore, to make the method safe with regard to a sufficient exchange of gases, it was arranged that the insufflation be interrupted, for a second or two, several times in a minute. This is the procedure now known under the name of "intratracheal insufflation"[2]—a practical, efficient method for intrathoracic surgery as well as for general anesthesia.

One of the possible dangers of this method is that of the entrance of foreign matter into the trachea, causing, possibly, an aspiration pneumonia. Since the tube keeps the glottis and epiglottis open, the secretions accumulating in the pharynx may conceivably enter alongside the tube into the trachea during an inspiration, or through an act of swallowing, or they may simply run into the

* Received for publication, April 15, 1911.

[1] Meltzer and Auer, *Jour. Exper. Med.*, 1909, xi, 622.

[2] Meltzer, *Med. Record*, 1910, lxxvii, 477.

larynx and trachea while the animal (or human being) is under deep anesthesia. This danger may be especially great when vomiting occurs while the tube is in the trachea. The belief of some writers[3] that vomiting can not take place while a tube keeps the glottis open, is, as we shall see later, not true, at least not to the extent assumed. We are not aware that this claim has ever been tested experimentally.

There is another assumption, which bears a special relation to this point, that has not been investigated experimentally; namely that aspiration into the trachea can not take place while the glottis is kept open by a tube. The rare occurrence of pneumonia in cases which have been intubated by the O'Dwyer method seems to support such an assumption. Kuhn,[4] the originator of the method of the peroral intubation, thoroughly believes this and quotes in its behalf a statement of one of the present writers[5] made in a discussion on intubation at the X International Congress held in Berlin in 1891. The remark reads as follows: "Sowohl Pneumonie, wie überhaupt alle Lungenerkrankungen, die durch Invasionen von infektiösen und indifferenten Fremdkörpern hervorgerufen werden, können bei offen stehender Glottis (wie bei der Intubation) gar nicht zustande kommen, weil das Hineingelangen von Fremdkörpern in die feinsten Bronchien nicht durch Aspiration, sondern durch einen rückläufigen Strom bewirkt wird, der entsteht, wenn bei geschlossener Glottis eine energische aktive Exspiration ausgeführt wird, so z. B. beim Husten oder bei der Aktion der Bauchpresse." From the above quotation it is evident that the writer did not intend to imply that no aspiration can occur, that is, that no foreign matter can enter the trachea and the larger bronchi as long as the glottis is kept open. It is only claimed that no aspiration pneumonia can develop, that is, that the aspirated foreign material can not be driven into the finest bronchi and alveoli, as long as the glottis is kept open. Even this latter point is only an hypothesis, the validity of which, however, need not be discussed

[3] Greene, *Med. News,* 1895, lxvii, 9; *Med. Record,* 1897, li, 465. Hubbard, *Med. Record,* 1897, li, 213.

[4] Kuhn, *Deutsch. Ztschr. f. Chir.,* 1905, lxxvi, 148.

[5] Meltzer, *Verhandl. d. X. internat. Med. Congr.,* 1892, iv, Abt. xii, 107.

here. For even if the assumption be correct, as long as foreign material can get into the trachea and bronchi at all, the danger of pneumonia remains, because on the withdrawal of the tube the normal activity of the glottis returns. On the other hand, there seems to be no valid reason for assuming, as Kuhn does, that no foreign material can enter the trachea as long as a tube is present within the larynx. This conclusion had, at the time it was made, no practical importance, since by Kuhn's method measures were taken to prevent the entrance of foreign material into the larynx alongside the tube. In recent communications, however, Kuhn[6] states expressly that such special measures are now omitted—"Es fällt jede Abdichtung weg." The question hence arises, by what mechanism, in the use of the method of peroral intubation, is the aspiration of foreign material prevented, and is it really prevented? For the method of intratracheal insufflation it is assumed that the practically continuous recurrent stream of air between the tube and the tracheo-laryngeal wall forms a safeguard against the entrance of foreign material into the respiratory tract. The numerous experiments on dogs, in which intratracheal insufflation was used, established the fact that such an invasion did not take place; the trachea was found in all cases examined to be perfectly free from extraneous material. However, the usual experience under normal conditions did not present a strong test since there was in the pharynx no unusual amount of foreign material; under intratracheal insufflation the animals never vomited. The same might be true also for the method of peroral intubation. However, some instances may present themselves in which there will be an unusual amount of mucus or blood, or the patient or the animal will vomit; the questions are then: What will happen to the respiratory tract under such circumstances? Will the return current of the insufflation method prove still to be a sufficient guard? And what guards against the invasion into the respiratory tract of these foreign materials in Kuhn's method of peroral intubation without the precautionary "Abdichtung"?

As regards intratracheal insufflation, some experiments, constituting severe tests, had already been made which were referred to very

[6] Kuhn, *Centralbl. f. Chir.*, 1908, xxxv, 788.

briefly in a previous communication.[7] But they were few in number and did not cover all the points concerned. The subject was therefore taken up again and a sufficient number of experiments made under various conditions to enable us to answer in a definite way the questions raised. The following is a brief account of these experiments.

INSUFFLATION AND EMESIS.

In one series of experiments the condition of the trachea and bronchi was studied after the animal was made to vomit. A few such experiments, as stated, had been made before with the insufflation method. This previous experience taught us a few facts, which now saved some preliminary work. It had been found previously that while the animal is receiving intratracheal insufflation it does not readily vomit, unless it has come entirely out of the anesthesia. Furthermore, in order to obtain a satisfactory result the stomach has to be filled first, preferably with an acid solution. Finally the vomiting has to be brought on by an active emetic, for instance by a subcutaneous injection of an effective dose of apomorphin. The present procedure of experimentation was as follows:

The animal was etherized, put on the board, the tube introduced into the trachea, and the ether continued by means of insufflation. Then a stomach tube was introduced and an acidulated solution, containing powdered charcoal in suspension, was poured into the stomach; whereupon the stomach tube was withdrawn. In some instances the stomach was filled before the insufflation tube was introduced. The ether was now discontinued (while the insufflation was continued with air), and apomorphin administered hypodermically. When vomiting occurred, the pharynx and mouth were not cleansed from the vomitus. Some time later ether was administered again, the trachea was exposed and clamped just beneath the larynx immediately on withdrawal of the insufflation tube, and the animal allowed to die from asphyxia. The trachea was now opened on the distal side of the clamp and examined for its contents and their reaction. The trachea should not be examined without previous clamping, because the very act of examination often causes an aspiration from the pharynx. For the same reason the findings above the clamp do not indicate the actual conditions prevailing before the opening of the trachea. In other experiments the animals were allowed to recover after the vomiting was over. They were killed later and the trachea and lungs were examined for the presence of charcoal.

We shall now present some abbreviated protocols illustrating the results of such experiments.

[7] Meltzer, *loc. cit.*

Experiment 1.—Yellow mongrel male dog; weight 10,000 grams. 11:10 a. m. Tube No. 17 (American) was introduced into the trachea, the end of the tube being half way between the larynx and sternum; insufflation started with ether. 11:20. The tube was introduced into the stomach and a suspension of charcoal in 250 c.c. of 0.7 per cent. hydrochloric acid was poured in. Ether was discontinued, and insufflation continued. 11:30. Animal coming out from anesthesia; 0.02 gram apomorphin injected subcutaneously. Vomiting promptly followed. 12 m. The trachea was clamped below the larynx. No particles of carbon were found; the reaction was faintly alkaline. There was a small amount of mucus in the bronchi.

Experiment 2.—Female mongrel; weight 6,800 grams. 2:45 p. m. Etherized, intubated, tube No. 15 (American), the end being just above the bifurcation; insufflation with ether. 2:55. 300 c.c. of a suspension of charcoal in water was introduced by stomach tube. Ether was stopped. 2:58. 0.017 gram apomorphin was given subcutaneously. 3:10. Animal vomiting, much retching. 3:15. Vomited a few c.c. 3:50. Animal killed by clamping trachea. No carbon was found in the trachea and bronchi. Pulmonary edema.

Experiment 3.—Black mongrel; weight 2,500 grams. 1:42 p. m. Ether, insufflation with ether. 100 c.c. of 0.7 per cent. hydrochloric acid with charcoal was poured into the stomach. 1:50. Ether was stopped, and insufflation continued. 1:54. Animal moving; 0.01 gram apomorphin was administered. Dog vomited. 2:35. The tube was removed and the dog released from the table; vomited twice later. Killed after 24 hours. Lungs and bronchi normal, no particles of coal.

In not a single instance in which insufflation was continued could the vomited material be discovered in the trachea. The animals which were permitted to survive remained well until they were subsequently killed.

TUBE IN TRACHEA WITHOUT INSUFFLATION.

In a number of experiments the insufflation was discontinued while the tube remained within the trachea, in order to ascertain whether the presence of the tube alone could prevent the entrance of the vomited material into the trachea, as is assumed by some writers.

Experiment 4.—Female dog; weight 4,000 grams. 1:21 p. m. Ether; tube No. 13 (American) was introduced into the trachea, the end being about 5 cm. below the larynx. 120 c.c. of 0.7 per cent. hydrochloric acid was introduced by tube into the stomach. 1:32. Ether and insufflation were stopped, the tube remaining in the trachea. 1:40. Animal moving; 0.015 gram apomorphin administered. 2:10. No vomiting yet; 0.015 gram apomorphin again administered. 2:15. Animal vomits dark brown acid liquid. 2:23. *Acid liquid comes from tracheal tube almost immediately after vomiting, and continues to come.* 2:40. The tube was removed and the dog released from the table; 5 c.c. of liquid obtained from the tube. Death occurred during the following night. The bronchi were partly filled with mucus and dark acid fluid. Areas of consolidation were present.

Experiment 5.—Female dog; weight 3,300 grams. 10:45 a. m. Ether, insufflation, the end of the tube being half way between the sternum and larynx; 90 c.c. acidulated suspension of charcoal was introduced by tube into the stomach. Ether and insufflation were stopped; the tube remained in place. 11:03. Dog moving; 0.01 gram apomorphin administered. 11:33. No vomiting yet; 0.01 gram apomorphin again administered. 11:37. Animal vomited *black fluid, 30 c.c. of which, mixed with particles of food and strongly acid, came from the tube.* 11:52. 7 c.c. of fluid of the same nature came in stream from the tube, although no fluid escaped from the mouth. 12:18. Animal died. Trachea and large bronchi contained vomitus with black particles; acid reaction. The left lower lobe showed pneumonic consolidation. The bronchi of this portion contained no charcoal.

Experiment 6.—Mongrel; weight 9,000 grams. 10:22 a. m. Ether; 250 c.c. aqueous suspension of charcoal introduced into the stomach by stomach tube, tracheal tube No. 11 (American), the end being midway between the sternum and larynx. 11:30. Ether and insufflation stopped, the tube remained in place. 10:37. 0.018 gram apomorphin given. 11:41. Animal vomited a large amount at once; 10 and 15 minutes later a few drops, not as acid as the vomitus, were obtained from the tube. 11:50. Animal killed by clamping trachea. Small specks of carbon found in trachea. Vomitus present in small amounts in bronchi of both lungs, and a good deal of carbon in the bronchi. Some pulmonary edema.

These experiments suffice to demonstrate unmistakably the fact that the simple presence of a tube within the trachea and the larynx does not prevent the entrance of foreign material into the respiratory tract. On the contrary, liquid may enter occasionally in such quantities as readily to escape again through the tube. Moreover, aspirated material may pass down into the bronchi and be the cause, either immediately or later, of serious consequences.

INSUFFLATION AND FILLING UP OF MOUTH AND PHARYNX.

In another series of experiments the test was made by filling up the mouth and pharynx with a suspension of charcoal, chiefly in an acid solution. The results were even more striking than in the previous experiments.

Experiment 7.—Brown and white dog; weight 6,100 grams. 11:15 a. m. Ether, intubation, tube No. 12 (American) inserted deeply in the trachea; insufflation and ether. 11:20. The mouth was filled with a suspension of charcoal in an acid solution. Until 12:10, the filling was repeated eight times. Insufflation continued during the entire experiment; the dog was out of ether for 22 minutes. 12:20. Trachea clamped. No black specks below the larynx.

Experiment 8.—Black and tan mongrel; weight 5,000 grams. 9:03. Intubation, tube No. 12 (American) inserted deeply; insufflation and ether. Until 10:25,

the mouth was filled eight times with an acid suspension of charcoal. Animal swallowed with difficulty. Insufflation continued throughout the experiment; dog fairly under ether. 10:25. Animal killed by clamping trachea. No carbon below clamp.

Experiment 9.—Yellow dog; weight 5,100 grams. 2:15 p. m. Intubation, tube No. 14 (American); insufflation and full ether throughout experiment; the mouth was filled many times with a charcoal suspension. 3:30. Animal killed by clamping trachea. No speck of carbon was found in the entire respiratory tract.

TUBE IN TRACHEA WITHOUT INSUFFLATION.

Experiment 10.—Black and white dog; weight 4,850 grams. 1:40 p. m. Intubation, tube No. 13 (American) inserted deeply within the trachea. 1:45. The mouth was filled with a suspension of charcoal in acid solution (30 c.c.), the dog swallowing most of the fluid at once. 1:53. A few drops of clear fluid came out from the end of the tube. The mouth was filled three times more, each time with 10 c.c.; the animal struggled and swallowed; no more drops came from the tube. 2:05. Etherized by gauze around the mouth. 2:10. Mouth filled. 2:14. Dog died. The bronchi were full of black specks; acute pulmonary edema.

Experiment 11.—Young dog; weight 3,100 grams. 2:00 p. m. Intubation, tube No. 9 (American) inserted deeply. No insufflation, ether anesthesia by gauze around the mouth. 2:03. Mouth filled with charcoal suspension, acid; most of the liquid was swallowed at once. 11:08. Mouth refilled; respiration became shallow. 11:13. Respiration ceased; the trachea was clamped and the lungs were examined immediately. Particles of charcoal of all sizes filled some of the fine and most of the large bronchi; extensive pulmonary edema.

Experiment 12.—Black and white male dog; weight 5,600 grams. 4:00 p. m. Ether, intubation, tube No. 13 inserted deeply. 4:12. The mouth was filled with charcoal suspension in acid solution, which was swallowed at once. Until 4:55 the mouth refilled six times. Fluid escaped from the tube. No dyspnea. 5:00. Animal killed by clamping trachea. From the trachea down to the finest bronchi large masses of carbon were present. Moderate pulmonary edema.

While in the animals which had insufflation throughout the experiments not a particle of carbon could be found in the respiratory tract below the clamp, in all the animals that had no insufflation the trachea and bronchi were filled with the charcoal masses. It seems that the anesthesia was a fatal factor in these animals. When the anesthesia was not deep, swallowing seemed to exert a beneficial influence by removing a great deal of the threatening material from the pharynx into the stomach. In the animals under the control of insufflation, the anesthesia exerted not the slightest unfavorable influence, although deglutition in these animals was not prompt.

CONCLUSIONS.

The foregoing experiments clearly justify the following conclusions:—

Intratracheal insufflation protects the respiratory tract very efficiently against any invasion from the pharynx. The filling up of the pharynx with extraneous material, whether it be from the stomach or from the mouth, brings no danger to the trachea and bronchi. This holds true even if the animal is under deep anesthesia.

On the other hand, the presence of a tube in the trachea or larynx without the protection of an effective recurrent air stream, definitely facilitates the entrance of foreign material from the pharynx into the trachea. Anesthesia, which removes the protective action of deglutition, greatly increases the danger from aspiration in these cases.

A FEW OBSERVATIONS ON THE ACTION OF SALVARSAN UPON THE IRRITABILITY OF NERVE AND MUSCLE.*

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In the literature upon salvarsan the question has been discussed whether or not salvarsan has a neurotropic action; whether, for instance, affections of the optic, auditory, and facial nerves, which sometimes occur after a salvarsan injection, are due to a neurotropic action of the drug or to a syphilitic manifestation. In this connection the question presented itself: What would be the effect upon nerve and muscle tissue if salvarsan solutions were applied directly to them? This problem was made the subject of a series of experiments performed upon nerve and muscle of the frog. The following is a brief presentation of the results.

Methods.—Leopard frogs (*Rana pipiens*) were used exclusively. The experiments were performed between February 10 and April 12, 1911. They fall into two groups: (A) perfusion experiments and (B) bathing experiments.

(A) PERFUSION EXPERIMENTS.

In these experiments a cannula was inserted in the abdominal aorta, directed distalward, and connected with a burette. The tendo Achillis was freed from its attachment and connected with a muscle lever to register the contractions of the gastrocnemius. From time to time during the perfusion, tests were made of the irritability of the sciatic nerve (indirect irritability) and of the gastrocnemius muscle (direct irritability). For this purpose single break induction shocks were used of such a strength as produced maximal contractions of the muscle.

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Both acid and alkaline solutions of salvarsan were used. In some cases the salvarsan was dissolved in a 0.6 per cent. saline solution; in others, in a Ringer solution the sodium chlorid content of which was isotonic with the tissue fluids of the frog (*i. e.*, 0.6 per cent.). The concentrations of salvarsan in saline or Ringer solution were 1:5000, 1:1000 and 1:500. Salvarsan is a dihydrochlorid and, in making the solutions termed acid, it was simply dissolved in 0.6 per cent. sodium chlorid without the addition of any sodium hydrate.

The alkaline solutions were made by adding one cubic centimeter of normal sodium hydroxid for each tenth gram of salvarsan—the amount of alkali just necessary to produce complete solution of the drug.

Results.—Several attempts were made to perfuse with an acid solution of salvarsan. These were invariably unsuccessful. A precipitate was formed at once when the salvarsan came in contact with the blood of the frog. It plugged the blood-vessels and stopped the solution when one half or one cubic centimeter was injected. Fresh frog's blood mixed with the salvarsan solution on a glass plate gave a heavy yellowish precipitate.

There was no such difficulty, however, with the alkaline solutions. Varying amounts of salvarsan were injected up to eighteen cubic centimeters. In no case was there the slightest alteration in the response of the muscle to direct stimulation. In the majority of cases the perfused salvarsan was equally without effect upon the indirect (nerve) irritability. There was infrequently a moderate reduction in the response through the nerve. This was never greater, however, than that which may be obtained by perfusing with sodium chlorid alone. In brief, then, it may be stated that alkaline solutions of salvarsan, given by perfusion in the amounts and concentrations mentioned, have no immediate effect whatever upon the irritability of nerve and muscle tissue in the frog.

(B) BATHING EXPERIMENTS.

In these experiments muscle-nerve preparations (gastrocnemius-sciatic) were immersed in the salvarsan solutions. Tests were taken

from time to time of the indirect irritability by means of single induction shocks of minimal strength. That is, the changes in irritability were gauged by changes in the threshold stimulus—the weakest stimulus which was capable of producing a response. At the time of stimulation the preparation was lifted out of its bath. The contractions were not registered graphically; they were simply judged by ocular inspection. In those cases in which the indirect irritability was finally abolished, tests were also taken of the *direct* muscle irritability. The following solutions of salvarsan were used for bathing:

(1) Salvarsan in saline solution (alkaline), 1:1000, 1:500, 1:250.
(2) Salvarsan in Ringer solution (alkaline), 1:1000, 1:500.
(3) Salvarsan in saline solution (acid), 1:500, 1:250.
(4) Salvarsan in Ringer solution (acid), 1:500.

The acid and alkaline solutions are the same as those used above for perfusion.

Results.—The effects of alkaline salvarsan baths will be given first. A solution of one part salvarsan in one thousand parts of 0.6 per cent. sodium chlorid produced only a slight reduction of indirect irritability after four hours.

A solution of salvarsan of the same strength in a Ringer solution was equally without injurious effect.

Alkaline solutions of salvarsan in saline solution (1:500, 1:250) caused almost complete abolition of indirect irritability after two and one half to five hours. During the first two hours the loss of irritability was slight. Toward the end it was more rapid.

The effects of acid solutions of salvarsan were as follows: an acid solution of salvarsan in saline solution (1:500) caused, in some cases, abolition, and in others, a reduction to about one half the previous irritability after five hours in the bath. During the first three hours no effect whatever was observable.

An acid solution of salvarsan in saline solution (1:250) had no effect after one hour, produced slight reduction of indirect irritability after two hours, and nearly an abolition of indirect irritability after two and a half hours.

In practically all cases in which the salvarsan finally caused an abolition of indirect irritability, it was found that the response, when the muscle was stimulated directly, was not better than that through its nerve. In other words both direct and indirect irritability decreased together.

Control experiments were performed by bathing muscle-nerve preparations in a 0.6 per cent. solution of sodium chlorid. It was found that sodium chlorid abolished indirect irritability in from six to eight hours or longer, while the direct muscle irritability still remained fairly good.[1]

It should be stated that all the solutions of salvarsan in Ringer solution, whether acid or alkaline, contained a more or less heavy yellow precipitate. The acid solutions of salvarsan in saline also developed gradually a similar precipitate. It appeared first immediately about the muscle and finally spread throughout the solution. Only the alkaline solutions of salvarsan in saline solution remained clear throughout the experiments. It is not impossible that this precipitation, by throwing some of the salvarsan out of solution, reduces the toxicity. It is well-known that either calcium or a carbonate will throw the salvarsan out of solution and this probably explains the precipitate formation in Ringer solutions. The precipitate formed in the acid saline bath was probably due to a decrease in acidity of the solution resulting from the presence of the muscle tissue.

CONCLUSIONS.

These experiments have shown that salvarsan is a comparatively inactive drug when applied directly to nerve and muscle tissue of the frog. In perfusion experiments with alkaline solutions, no detrimental action whatever was to be seen either upon direct or indirect irritability. In bathing experiments, in which the concentration of the salvarsan in the various solutions was surely much higher than that in which it reaches the peripheral tissues in the human subject through the circulation, the loss of irritability occurred only after a long period of exposure to the drug.

[1] This statement applies to the gastrocnemius and not to the sartorius which, according to Locke and others, loses its indirect irritability very quickly in a sodium chlorid solution.

THE BACTERIOLOGY OF SPUTUM IN COMMON NON-TUBERCULOUS INFECTIONS OF THE UPPER AND LOWER RESPIRATORY TRACTS, WITH SPECIAL REFERENCE TO LOBAR AND BRONCHO-PNEUMONIA.*

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In a concise and interesting paper, Kitasato (1) discusses the bacteria in the sputum from pulmonary tuberculosis and gives the simplest and most reliable method of isolating bacteria from sputum containing several organisms. The cultural investigations here recorded were stimulated by the paper of Kitasato and, in 1905, by that of Schottmüller (2); and the article of Norris and Pappenheimer (3) called our attention to the fact that post-mortem bacterial examinations of material from the respiratory tract are not reliable, and showed that careful sputum examinations might be of considerable value in prognosis and also in treatment, now that specific therapy is being developed.

The examinations of infections of the respiratory tract here recorded are for the years 1903 to 1910, and were made upon patients visiting the Dispensary of Cornell University Medical College, upon those admitted to the wards of the Second Division of Bellevue Hospital, and upon a few private patients. The earlier examinations were made by Dr. Hastings and Dr. Mortimer Warren, the later ones by Dr. Armstrong and Dr. Niles. The study of the sputum throughout a series of years has shown a marked yearly variation in the types of infecting organism; so that it is safe to conclude that the observations on one set of cases for a few months have little weight in the determination of the infectivity of an

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organism for the respiratory tract and of the frequency of the association of any one germ with certain clinical symptoms and signs.

THE VALUE OF THE LITERATURE UPON THE BACTERIOLOGY OF THE RESPIRATORY TRACT.

The literature regarding the bacteriology of the healthy respiratory tract below the larynx is voluminous, as the subject has been extensively investigated in man and animals. Nevertheless, in most of the work two serious errors are found which vitiate the strength of the conclusions.

The first error is, that cultures have usually been taken post-mortem, and the findings assumed to represent the bacterial flora ante-mortem. That this is fallacious was shown by the investigations of von Besser (4), and more recently by Norris and Pappenheimer who state: "It follows logically from the results obtained in this experiment that the cultural findings after death are no guide to the bacterial contents of the lungs during life, and that any deductions made from such findings are unreliable and deceptive."

The second error is self-evident and consists in drawing conclusions from examinations of sputum that has been collected without regard to the contamination that must take place unless precautions are observed while the sputum is passing through the pharynx and mouth.

It seems to have been generally assumed that the bronchi and alveoli are normally infected, and such authorities as Baumgarten (5) and Hoffmann (6) state that such is the case. Baumgarten, however, offers no proof, and Hoffmann merely quotes Pansini as having found several species of bacteria in healthy lungs, but gives no details. Dürck (7), who examined healthy lungs of man and animals, is quoted as believing in normal lung infection, but as his work was done post-mortem, his conclusions can not be accepted without reservation.

RESULTS OF EARLIER INVESTIGATIONS IN SPECIAL DISEASES.

It seems hardly worth while to review much of the literature on the bacteriology of respiratory diseases because of the variations in technique and the lack of precautions observed by the investi-

gators. Some of the more recent and important work will, however, be noted.

In acute catarrhal inflammations of the nose, White (8) reports that *B. coryzæ segmentosus*[1] was present in fifty out of fifty-six cases examined, and he regards it as the most frequent cause. At the same time (1906), Allen (9) apparently regarded the bacillus of Friedländer as more important. In 1908, however, he reported the bacteriology of forty-two "colds" occurring in London during the previous three years. This time he found *B. influenzæ* alone in 2.4 per cent., *B. Friedländeri* in 19.0 per cent., *B. coryzæ segmentosus* in 26.2 per cent., and *Micrococcus catarrhalis* in 28.6 per cent. of the cases. He concluded that any of the above mentioned organisms might cause acute nasal catarrh; that subacute catarrh is commonly caused by the bacillus of Friedländer or by *Micrococcus catarrhalis*, but rarely by *B. influenzæ*, or by *B. coryzæ segmentosus;* and chronic catarrh by Friedländer's bacillus only.

In tracheal catarrhs, Allen (10) concludes that *B. influenzæ* or *Micrococcus catarrhalis* are usually found, the bacillus of Friedländer exceptionally. *Micrococcus paratetragenus* was not found by him, although it has been reported in trachitis by Bezançon and De Jong (11), and by Benham (12).

As inflammation of the bronchial mucous membrane often accompanies specific diseases and often arises independently, it seems well, as Marfan (13) has suggested, to adopt a bacteriological classification. Marfan makes two general groups. Group 1 is due to specific infections (influenza, pertussis, measles, diphtheria, anthrax, plague, tuberculosis, variola, malaria, glanders, and syphilis), and group 2 is due to non-specific infections. These he believes arise chiefly from pneumococci and streptococci.

B. influenzæ is constantly found in the bronchitis of epidemic influenza, and Kretz (14), Washbourn (15), and Lord (16) have called attention to its frequency in the respiratory tract in diseases other than epidemic influenza. Davis (17) and Wollstein (18) report an organism, which appears to be identical with the influenza bacillus, as almost constantly present in the sputum of pertussis. Goldie (19) has seen two fatal cases of general bronchitis in which no membrane formed, but from the secretions of which pure cultures of the diphtheria bacillus were obtained.

Many forms of microörganisms have been found in exudates of non-specific bronchitis, and the infection is usually a mixed one, two, or three, or more varieties being present. Undoubtedly some are often secondary invaders, but pathogenic properties have been attributed to many.

Pneumococci and streptococci alone, or associated with each other or with other bacteria, have been most frequently reported. Ritchie (20, 21), who has thoroughly reviewed the literature of the subject, examined forty-nine cases and found these two organisms most commonly. His work, however, like that of many other investigators, is open to the criticism that his cultures, though made from the smaller bronchioles, were taken post-mortem, and may, therefore, be misleading. Among those who found pneumococci predominating are von Besser

[1] We have found *B. coryzæ segmentosus* once in the sputum of chronic bronchitis in a dog.

(22) and Barthel (23); while Kruse, Pansini and Pasquale (24), Bouchard (25), Claisse (26), Queyrat (27), Cassaët (28), Hoffmann (29), and Forchheimer (30) found streptococci in abundance. Babes and Popesco (31) regard *Staphylococcus aureus* as more important, and Durante (32) reports staphylococci in pure culture in four cases. Patton (33) found streptococci and staphylococci in most cases.

An important recent report is that of Pollak (34), who examined seventy-three cases and found twelve varieties of bacteria, including *B. coli communis, B. lactis aerogenes,* and *B. proteus.*

Ghon and Pfeiffer (35) and Sederl (36) have emphasized the importance of *Micrococcus catarrhalis* in acute bronchitis. Others which seem occasionally to be the specific cause are, *B. Friedländeri, Micrococcus tetragenus,* and several saprophytes.

The frequency of *B. influenzæ* in bronchiectasis has been emphasized by Boggs (37) who reports five cases, in three of which the bacilli were in pure culture, and present with pneumococci in the other two cases. Sections from two fatal cases revealed *B. influenzæ* deep in the walls of the bronchi and mostly intracellular.

Lobar (Croupous) Pneumonia.—The *Diplococcus lanceolatus* (pneumococcus) has been so constantly demonstrated in the exudate of acute lobar pneumonia that it is generally regarded as the specific etiologic agent. This view receives corroboration by the demonstration of an accompanying pneumococcemia in a large proportion of cases, Prochaska (38) having found it in all of fifty cases, and Rosenow (39) in 132 out of 145 cases, both believing the blood invasion to be a constant condition. It is probable that other organisms may occasionally induce a pneumonia which can not be clinically or pathologically differentiated from that caused by the pneumococcus.

This property has been long attributed to Friedländer's bacillus, and Schottmüller (2) has described five fatal cases of apparently typical lobar pneumonia, also one non-fatal empyema from which he recovered *Streptococcus mucosus* in pure culture. Secondary invasion by other microörganisms is common, and it seems likely that these may at times influence the course of the disease.

Streptococcus pyogenes is regarded as the most common associate of the pneumococcus, although *Staphylococcus pyogenes aureus, B. influenzæ, B. diphtheriæ,* and *Micrococcus catarrhalis* are not infrequent.

Broncho-Pneumonia.—In this form of pneumonia no specific organism has been determined. The infection is usually a mixed one, but in the primary form the pneumococcus alone or associated with others is the bacterium found most frequently. Wollstein (40) took cultures post-mortem from infants and found pneumococci in 81 per cent. of thirty-three cases, and in pure culture in 41 per cent. Two cases showed streptococci alone. *Staphylococcus aureus* was recovered in pure culture from two cases, and in one case it was found in association with *B. coli communis.*

Pfeiffer (35) and Sederl (36) report *Micrococcus catarrhalis* in pure culture from broncho-pneumonia as well as from bronchitis.

In the secondary form, mixed infections are again the rule, all the above mentioned organisms having been found in association with the specific cause of the primary disease.

Sterility of Upper and Lower Respiratory Passages.—In order that the bacteriological findings in exudates from the respiratory tract may be of value in representing the specific cause of an inflammation, it is necessary to establish the validity of two propositions: (1) that most of the normal respiratory tract, particularly its lower part, is sterile, and (2) that it is possible to collect and handle exudates in such a manner that bacterial contamination from the normally infected parts may be obviated.

The Nasal Mucosa.—The evidence regarding the sterility of the healthy nasal mucosa is conflicting. This is principally because of the difficulty in deciding whether or not a nose is really normal, but partly because every precaution may not have been taken in collecting exudates. All agree that large numbers of atmospheric bacteria are constantly found in the vestibula of the nares and on the vibrissæ, which act as filters, and contamination from these sources is likely, unless precautions are observed. The careful work of Fraenkel (41) and Löwenberg (42), who found no bacteria in the interior of healthy noses, is, however, convincing. Others who have found most healthy noses sterile are Hildebrandt (43), Lermoyez and Wurtz (44), and Thomson and Hewlett (45). Lewis and Turner (46) conclude that healthy accessory sinuses are also probably sterile.

The Mouth and Pharynx.—The mouth and pharynx, whether healthy or diseased, are, of course, commonly infected with the atmospheric microörganisms and not infrequently with bacteria, which are commonly pathogenic, notably pneumococci (Hiss, 47).

The Respiratory Tract Below the Larynx.—Many observers, however, have found the air passages of healthy individuals from the glottis down to be usually sterile, or at most to contain very few bacteria. Among these may be mentioned Babes (48), Thomson and Hewlett (49), Müller (50), Barthel (23), Klipstein (51), and Jundell (52).

Jundell carried out a particularly interesting investigation, obtaining mucus from the tracheas of forty-three healthy human beings by means of a special instrument which he devised. That these observations are probably correct is borne out by the following conclusions of Ritchie (20):

(*a*) All bacteria contained in inspired air are probably withdrawn in the winding upper air passages.

(*b*) The nasal mucous membrane possesses marked bactericidal properties.

(*c*) Inspired air remains mostly in the upper portions of the bronchial tract, seldom reaching the alveoli.

(*d*) Expired air contains no bacteria.

(*e*) Bacteria when introduced into the bronchi or lungs of healthy animals soon die.

We agree with Krehl (53) and Ritchie (20), both of whom conclude that the air passages of healthy individuals below the glottis are usually sterile.

Exudates formed in portions of the respiratory tract that are normally sterile may be collected and treated in a way that will prevent contamination. The anterior nares are readily disinfected and this should be done before collecting exudates from the nose. Contamination in the mouth and pharynx is avoided by the method of Kitasato (1) who collected in a sterile dish sputum from deep coughing, after which he picked a small piece from the center, washed it in sterile water, and then planted it on proper media. If previous to this collection the mouth and pharynx are washed with salt solution or a mild antiseptic, there is little liability of contamination. The more elaborate method of Löwenstein (54) seems unnecessary.[2]

We assume, therefore, that when the proper precautions are observed, the bacteria found in an exudate from the respiratory tract are the specific cause of the inflammatory process which produced the exudate.

METHODS EMPLOYED AND NOMENCLATURE ADOPTED.

The technique used was that suggested by Kitasato. The mouth and pharynx were rinsed with sterile water or salt solution and then the sputum from a single expectoration was collected in a bottle that had been sterilized by boiling. From the sputum thus obtained the portion to be examined was selected and washed in 0.8 per cent. sterile salt solution.

[2] Löwenstein collected in a sterile dish early morning sputum after having the patient wash the mouth with a thymol mouth-wash after meals and at night, for the three preceding days. He then washed the pieces of sputum in hot water and followed this with a quick washing in a 3 per cent. solution of hydrogen peroxid.

A *clean sputum, i. e.,* one containing only two or three types of bacteria and free from buccal squamous cells, and a *dirty sputum, i. e.,* one containing a varied bacterial and fungoid flora and buccal squamous cells, are readily recognized on microscopical examination.

A dirty sputum is not suitable for bacterial examination and should be discarded for a second or third clean specimen from the same patient.

The more carefully the clean specimens are selected, washed, and handled, the more frequently does one obtain pure, unmixed cultures. If collected and handled properly, washing may be dispensed with. Inoculations should be made on tube-slants of plain and glycerine agar and in plain broth, and from the first tube of broth a platinum loop full or two should be transferred to a second tube of broth, from which streaks should be made upon agar plates. Except when *Micrococcus catarrhalis* is present, a mixed flora may, as a rule, be easily separated on the first trial. For differentiation, the usual bacteriological methods are to be followed, namely, the making of cultures upon glucose and other carbohydrate media, upon hemoglobin media, and upon blood serum. Rarely, anaerobic cultures may be necessary.

Whenever the stained smears from the sputum have shown small gram negative bacilli which might be *Bacillus influenzæ,* cultures have been made directly upon fresh hemoglobin media.

Nomenclature.—The term micrococcus has been used for the various cocci, including staphylococci, *e. g., Micrococcus aureus, Micrococcus albus, Micrococcus catarrhalis.* Streptococci[3] have been classed, wherever the proper differentiation has been carried out, as *Streptococcus pyogenes* (*longus et erysipelatous*), *Streptococcus hemolysans* (*longus seu brevis*), *Streptococcus viridans or mitior* (*longus seu brevis*).

The *Streptococcus mucosus* and pneumococcus have been considered as more closely related to each other than to the three types of streptococci mentioned.

The bacillus of Friedländer and the other types of closely related

[3] Schottmüller (55) classifies the three groups of streptococci as: (1) *Streptococcus longus et erysipelatous,* (2) *Streptococcus mitior seu viridis,* and (3) *Streptococcus mucosus.*

gram negative, encapsulated, aerobic bacilli have been classed under *Bacillus mucosus capsulatus.*

The organisms found were as follows: *Streptococcus pyogenes, Streptococcus hemolysans,* and *Streptococcus mitior,* pneumococcus, *Micrococcus catarrhalis, Micrococcus tetragenus, Micrococcus aureus, Micrococcus albus, Micrococcus citreus, Streptococcus mucosus, Bacillus mucosus capsulatus* (which includes *Bacillus Friedländeri*), *Bacillus influenzæ, Bacillus fluorescens, Bacillus pyocyaneus, Bacillus coli,* and *Bacillus acidi lactici.*

GROUPS OF NON-TUBERCULOUS DISEASES STUDIED.

Our 183 cases have been divided into nine groups. The first two of these deal with the upper respiratory tract, the larynx, trachea, and larger bronchi, and the other seven with the lower tract, the smaller bronchi, vesicles, and pleuræ.

Group 1 consists of twelve cases; *i. e.,* acute laryngitis (2), acute trachitis (9), and chronic trachitis (1).

Group 2 contains twenty-seven cases of grippe, nineteen of these with or without coryza (including influenza), and eight with no diagnosis other than grippe, probably acute bronchitis (including influenza).

Group 3 embraces nine cases of acute bronchitis.

Group 4 consists of forty-eight cases of chronic bronchitis.

Group 5 embraces sixty-six cases of lobar pneumonia. In only twenty-three of these were pneumococci found in culture.

Group 6 includes twelve cases of bronchial pneumonia; three were typical, and nine atypical, the so-called grippe or influenza pneumonia.

Group 7 contains two cases of bronchiectasis.

Group 8 consists of two cases of asthma.

Group 9 includes five cases of pleuritis, or pleurisy, with no other diagnosis.

SUMMARY OF CULTURES FROM GLOTTIS DOWN, IN ORDER OF FREQUENCY.

Organisms	No. of positive sputa.	Percentage of occurrence
M. catarrhalis	71	21.0
M. aureus	52	15.0
Streptococci	50	14.0
Pneumococcus	45	13.0
		= 63.0

M. tetragenus	35	10.0	
M. albus	21	6.0	
B. Friedländeri	13	4.0	
B. influenzæ	6	1.5	
			= 21.5
Streptococcus mucosus	5	1.5	
M. citreus	4	1.25	
B. fluorescens	4	1.25	
			= 4.0
B. coli	3	1.0	
B. pyocyaneus	3	1.0	
B. proteus	3	1.0	
Oidium albicans	2	— 1.0	
Hay bacillus	2	— 1.0	
B. acidi lactici	1	+ .5	
			= 5.5
Unidentified organisms	11	3.0	
Total No. of isolations =	331		= 97.0
Sputa culturally negative	10	3	
Total No. of cultures =	341		100

SUMMARY OF CULTURES BY YEAR.

	Year.	Number.	Most common.	No. of times isolated.	Per cent.
1.	1903–04	34	Pneumococcus	8	23.5
			M. catarrhalis	7	20.5
			M. aureus	5	14.5
			M. tetragenus	3	9.0
			M. albus,	3	9.0
			Other organisms and negative cultures	8	23.5
2.	1904–05	63	*M. catarrhalis,*	18	29.0
			Streptococci	9	14.0
			M. tetragenus	8	13.0
			Pneumococcus	6	9.5
			M. aureus	6	9.5
			Other organisms and negative cultures	16	25.0
3.	1905–06	31	*M. catarrhalis*	5	16.0
			M. tetragenus	4	13.0
			M. aureus	4	13.0
			Pneumococcus	3	10.0
			Other organisms and negative cultures	15	48.0
4.	1906–07	23	*M. tetragenus*	5	22.0
			Streptococci	4	17.5
			Pneumococcus	3	13.0
			Streptococcus mucosus	3	13.0
			Other organisms and negative cultures	8	34.5

5.	1907–08	84	*M. catarrhalis*	17	20.5
			Streptococci	16	19.5
			M. tetragenus	12	14.0
			Pneumococcus	12	14.0
			Other organisms and negative cultures	27	32.0
6.	1908–09	55	Streptococci	12	22.0
			Pneumococcus	10	19.0
			M. catarrhalis	9	17.0
			M. aureus	8	15.0
			Other organisms and negative cultures	16	27.0
7.	1909–10	51	*M. aureus*	18	35.5
			M. catarrhalis	15	29.5
			Streptococci	6	11.5
			Pneumococcus	3	6.0
			Other organisms and negative cultures	9	17.5
Total number of cultures				341	

THE ORGANISMS PRESENT IN THE CLINICAL GROUPS.

Group 1 (*Laryngitis and Trachitis*).—The organisms most frequently isolated were: *Micrococcus catarrhalis* (7 times) and *Micrococcus aureus* (6 times). Pure cultures of *Micrococcus aureus* were obtained in 3 cases, and *Micrococcus catarrhalis* in 1.

Group 2 (*Grippe and Influenza, probably with Acute Bronchitis*).—In this group *Micrococcus catarrhalis* was the organism most frequently isolated (15 times), streptococci[4] were found in 7 cases, and the pneumococcus in 7. *Bacillus influenzæ* was present in 7, and *Micrococcus tetragenus* in 6 cases. In 8 instances the sputa gave pure cultures: *Micrococcus catarrhalis* in 4, *Micrococcus albus* in 2, *Micrococcus tetragenus* in 1, and streptococcus in 1.

Group 3 (*Acute Bronchitis*).—*Micrococcus catarrhalis* was the organism most commonly found, being isolated 6 times. Two of the cases gave pure cultures, one of *Micrococcus catarrhalis* and one of pneumococcus.

Group 4 (*Chronic Bronchitis*).—The organisms isolated from this group and their frequency were as follows: *Micrococcus aureus* (17), streptococci (15), *Micrococcus catarrhalis* (13), *Micrococcus*

[4] The types of streptococci have not been separated for this report. From a study of the types isolated from specimens in this laboratory they have been classed as (*a*) *Streptococcus pyogenes* (*longus et erysipelatous*), (*b*) *Streptococcus hemolysans* (*longus seu brevis*), and (*c*) *Streptococcus viridans seu mitior* (*longus seu brevis*). Our classification is a modification of that of Schottmüller.

tetragenus (10), pneumococcus (8), *Streptococcus mucosus* (4), *Bacillus influenzæ* (1). Pure cultures were obtained in 18 cases, as follows: *Micrococcus aureus* (7), *Micrococcus albus* (2), pneumococcus (2), *Micrococcus catarrhalis* (1), *streptococci* (1), *Streptococcus mucosus* (1), *Micrococcus tetragenus* (1), *Bacillus coli* (1), *Bacillus pyocyaneus* (1), Friedländer's bacillus (1).

Group 5 (*Lobar Pneumonia*).—There were twenty-three cases with pneumococci and forty-three cases with no pneumococci in the sputum. These results do not represent the proportion of cases in which the pneumococcus occurs in lobar pneumonia, for many of the cultures were taken because the clinical manifestations of the disease were atypical.

Of the twenty-three sputa giving pneumococci, nine revealed them in pure culture. The organisms were as follows: *Micrococcus catarrhalis* in 7 cases, streptococci in 3, *Micrococcus aureus* in 3, *Micrococcus tetragenus* in 5, *Micrococcus albus* in 5, Friedländer's bacillus in 1, *Bacillus proteus* in 1, *Micrococcus citreus* in 1, and unidentified organisms in 6.

From the forty-three sputa without pneumococci in cultures, the following organisms were most frequently recovered: streptococci in 14, *Micrococcus catarrhalis* in 16, *Micrococcus aureus* in 11, *Micrococcus tetragenus* in 8, Friedländer's bacillus in 4, and *Bacillus coli* in 2 cases. The pure cultures from these cases were 18 in number, as follows: *Micrococcus aureus* in 6, streptococci in 5, *Micrococcus catarrhalis* in 4, *Bacillus coli* in 1, *Bacillus proteus* in 1, and *Micrococcus citreus* in 1.

Fresh smears were made from every exudate, and in only four of the above were capsulated cocci in pairs observed. In four of the negative cases the pneumococcus was found neither in cultures nor smears. In three others the cultures were reported negative for pneumococci and smears were positive for pneumococci. We consider that a few scattered pneumococci might be found in smears from buccal cavity contamination, while the cultures might be negative. The opposite of this, *i. e.*, that smears might be negative and cultures positive, is untenable, and our results bear this out. The hay bacillus in one case was a contamination.

From lobar pneumonia the most common isolations were: the

pneumococcus in 23 instances, *Micrococcus catarrhalis* in 23, streptococci in 17, *Micrococcus aureus* in 14, *Micrococcus tetragenus* in 13, *Micrococcus albus* in 7, *Bacillus Friedländeri* in 5, *Bacillus proteus* in 2, *Bacillus coli* in 2, *Bacillus fluorescens* in 1, *Micrococcus citreus* in 3, and unidentified organisms in 6. The total number of isolations from lobar pneumonia was 110.

Group 6.—In broncho-pneumonia the organisms most frequently isolated were: *Micrococcus catarrhalis* in 5 cases, streptococci in 4, *Micrococcus aureus* in 14, pneumococcus in 2, and *Bacillus Friedländeri* in 2. Pure cultures were obtained from seven cases, as follows: *Micrococcus aureus* from 3, *Bacillus Friedländeri* from 2, *Bacillus pyocyaneus* from 1, and pneumococcus from 1.

Group 7.—One exudate from bronchiectasis revealed a pure culture of the streptococcus, and from the other were isolated *Micrococcus albus* and *Bacillus proteus.*

Group 8.—From the bronchial asthma group, *Micrococcus catarrhalis* and the *Micrococcus tetragenus* were each isolated once in pure culture.

Group 9.—The five cases of pleurisy in this group could not be proven tuberculous. The production of sputum is evidence of some condition other than pleurisy, but no other diagnosis appeared in the records. The pneumococcus was isolated in 3 cases; the micrococcus in 2; *Bacillus Friedländeri* in 1; and *Bacillus fluorescens* in 1. One case gave a pure culture of pneumococcus.

The yearly variation is noteworthy, and also the fact that it can not be accounted for by variations in the number of examinations made in the different years. The organisms which were first in frequency, and the winter seasons from 1903 to 1910 inclusive during which they were first, are as follows: *Micrococcus catarrhalis* (1904–05), *Micrococcus catarrhalis* (1905–06), *Micrococcus catarrhalis* (1907–08), pneumococcus (1903–04), streptococci (1908–09), *Micrococcus aureus* (1909–10), *Micrococcus tetragenus* (1906–07); and those second in frequency were: pneumococcus (1908–09), streptococci (1904–05), streptococci (1906–07), streptococci (1907–08), *Micrococcus catarrhalis* (1903–04), *Micrococcus catarrhalis* (1909–10), *Micrococcus tetragenus* (1905–06).

SUMMARY.

1. In our examinations, only 38 per cent. of the infections of the respiratory tract below the glottis were pure, and this percentage was reached only by carefully following Kitasato's method of handling sputa.

2. Lobar pneumonia may produce sputum free from pneumococci, and may undoubtedly be caused by organisms other than the pneumococcus.

3. There is found a marked yearly variation in the organisms which excite inflammation of the respiratory tract.

4. *Micrococcus catarrhalis* is usually considered a common secondary invader; but it may, and probably frequently does, assume pathogenic properties.

We extend our appreciation particularly to Dr. Thomas H. Evans for his valuable assistance in verifying the records and compiling the tables.

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Tabulated Summary of Isolations According to Season and Incidence.

Group numbers.	Groups and subdivisions.	No. of cases.	Year.	Streptococcus.	Pneumococcus.	M. catarrhalis.	M. tetragenus.	M. aureus.	M. albus.	M. citreus.	Streptococcus mucosus.	B. mucosus capsulatus.	B. influenzæ.	B. fluorescens.	B. proteus.	B. pyocyaneus.	B. coli.	B. acidi lactici.	Unidentified.	Oidium.	Pneumococcus in smears.
I	Laryngitis (acute)	1	1903–04	....	+	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
		1	1905–06	+	....	+	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....
I	Trachitis (chronic)	1	1909–10	+	....	+	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....
I	Trachitis (acute)	1	1904–05	....	....	+	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....
		1	1907–08	....	....	....	....	+	+	....	....	....	....	....	....	....	....	....	....	....	....
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II	Grippe (with or without coryza)	1	1903–04	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	....	....
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II	Grippe (with no other diagnosis)	1	1903–04	....	+	+	....	+	....	....	....	....	+	....	....	....	....	....	....	....	....
		1	1904–05	....	....	+	....	+	+	....	....	....	+	....	....	....	....	....	....	....	....
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		1	1907–08	....	+	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....
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III	Bronchitis	1	1903–04	....	+	+	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....
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II	Grippe (with no other diagnosis)	1	1903–04		+	+		+				+								
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III	Bronchitis	1	1903–04		+	+	+													
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IV	Bronchitis (chronic)	1	1903–04								+									
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TABULATED SUMMARY OF ISOLATIONS ACCORDING TO SEASON AND INCIDENCE.—CONTINUED.

Group numbers.	Groups and subdivisions.	No. of cases.	Year.	Streptococcus.	Pneumococcus.	M. catarrhalis.	M. tetragenus.	M. aureus.	M. albus.	M. citreus.	Streptococcus mucosus.	B. mucosus capsulatus.	B. influenzæ.	B. fluorescens.	B. proteus.	B. pyocyaneus.	B. coli.	B. acidi lactici.	Unidentified.	Oidium.	Pneumococcus in smears.
V	Pneumonia (lobar, with no pneumococci in sputum)	1	1904–05	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
		1	1904–05	....	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
		1	1905–06	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+
		1	1905–06	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+
		1	1906–07	+	....	....	....	....	....	....	....	....	+	....	+	....	....	....	....	....	+
		1	1906–07	+	....	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	+
		1	1907–08	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+
		1	1907–08	+	....	+	+	....	+	....	....	....	....	....	....	....	....	....	....	....	+
		1	1907–08	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	?
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		1	1909–10	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+
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		I	1907–08	....	....	....	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....
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		I	1909–10	....	....	+	....	....	....	....	....	....	....	....	....	....	+	....	....	....	−
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		I	1909–10	....	....	+	....	+	....	....	....	....	....	....	....	....	....	....	....	....	−
		I	1909–10	....	....	....	....	+	....	....	....	....	....	....	....	....	....	....	....	....	−
		I	1909–10	....	....	....	....	....	....	....	....	....	....	....	....	....	+	....	....	....	−
		I	1909–10	....	....	....	....	....	+	....	....	....	....	....	....	....	....	....	....	....	−
		I	1909–10	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	−
		I	1909–10	....	....	....	....	....	....	....	....	....	....	....	....	....	+	....	....	....	....
V	Pneumonia (lobar, with pneumococci in sputum)	I	1903–04	....	+	+	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....
		I	1903–04	....	+	+	....	....	+	+	....	....	....	....	....	....	....	....	....	....	+
		I	1904–05	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+
		I	1906–07	....	+	....	....	....	+	....	....	....	....	....	....	....	....	....	....	....	+
		I	1907–08	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+
		I	1907–08	....	+	+	+	....	....	....	....	....	....	....	....	....	....	....	....	....	+
		I	1907–08	....	+	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+
		I	1907–08	....	+	+	+	....	....	....	....	....	....	....	....	....	....	....	....	....	+
		I	1907–08	+	+	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	+
		I	1907–08	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+
		I	1907–08	+	+	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....
		I	1907–08	....	+	+	....	....	+	....	....	....	....	....	....	....	....	....	....	....	....
		I	1907–08	+	+	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
		I	1907–08	....	+	....	....	+	+	....	....	....	....	....	....	....	+	....	....	....	....
		I	1907–08	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
		I	1908–09	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
		I	1908–09	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+
		I	1908–09	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
		I	1908–09	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
		I	1908–09	....	+	....	+	....	....	....	....	....	....	+	....	....	....	....	....	....	+
		I	1909–10	....	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
		I	1909–10	....	+	....	....	+	....	....	....	....	....	....	....	....	....	....	....	....	+
		I	1909–10	....	+	....	....	....	+	....	....	....	....	....	....	....	....	....	....	....	+

VI	Pneumonia (bronchial, typical)	1	1904–05	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	+
		1	1908–09	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	−
		1	1908–09	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	−
VI	Pneumonia (bronchial, atypical)	1	1903–04	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.
		1	1904–05	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
		1	1905–06	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
VI	Pneumonia (bronchial, atypical, or typhoid)	1	1904–05	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	+
		1	1905–06	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	+
VI	Pneumonia (bronchial, atypical, and sepsis)	1	1908–09	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		1	1907–08	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	−
		1	1908–09	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	−
		1	1908–09	+	.	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
VII	Bronchiectasis	1	1904–05	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		1	1907–08	.	.	.	.	.	+	.	.	.	.	.	+	.	.	.	.	.	.
VIII	Asthma	1	1903–04	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		1	1904–05	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
IX	Pleuritis (with no other diagnosis)	1	1905–06	.	.	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
		1	1905–06	.	.	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.
IX	Pleurisy (with emphysema)	1	1904–05	+	.	.	.	.	.	.	.	+	.	+	.	.	.	.	.	.	.
IX	Pleurisy (with effusion)	1	1908–09	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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THE RESISTANCE OF THE END-PIECE AND MID-PIECE OF COMPLEMENT INACTIVATED BY DISTILLED WATER.*

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In 1907, Sachs and Teruuchi[1] reported that if fresh guinea pig serum were appropriately diluted with distilled water (optimum relationship, 1 part serum to 8 to 9 parts distilled water), and incubated for an hour or more in a water bath at 37° C., on the restoration of isotonicity, complement activity no longer took place. The absence of hemolysis they attributed to a permanent destruction of complement, produced, they assumed, by a ferment first becoming active in the diluted guinea pig serum. More recently, however, Sachs and Bolkowska[2] have observed that at times complement activity may be restored to such inactivated serum on the addition of either fresh guinea pig end-piece or guinea pig mid-piece. This latter observation, taken in connection with recent work[3] on the resistance of the globulin fraction of guinea pig serum inactivated by heat, suggested that perhaps the resistance of one or possibly of both complement fractions might likewise be directly demonstrated in serum rendered inactive by dilution with distilled water.

The resistance of the globulin fraction was first examined. The method employed is as follows:

A series of centrifuge tubes, each containing one cubic centimeter of guinea pig serum to nine parts of distilled water, was incubated in a water bath at 37° C. for one and one quarter hours. Two of these tubes were then made isotonic by the addition of sodium chlorid and used as controls, their contents being directed against

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[1] Sachs and Teruuchi, *Berl. klin. Wchnschr.*, 1907, xliv, 467, 521, 602.

[2] Sachs and Bolkowska, *Ztschr. f. Immunitätsforsch.*, 1910, vii, 785.

[3] Marks, *Jour. Exper. Med.*, 1911, xiii, 590.

sheep corpuscles laden with ten amboceptor units. It was found that complement treated with distilled water could not be absolutely inactivated. Therefore only such complement was used in the experiments, as gave, in the amounts of 0.3 or 0.2 cubic centimeter, only a faint tinge to the supernatant fluid after incubation for five or six hours in a water bath at 37° C., while the supernatant fluid in the tube containing 0.1 cubic centimeter remained perfectly clear. The globulin fraction was next precipitated from the remaining tubes, washed three times with distilled water, and dissolved in suitable amounts of physiological salt solution. Against diminishing amounts of such globulin fraction, a constant amount of fresh end-piece was then directed (table I). It may be added that both the hydrochloric and carbon dioxid methods were used to obtain the complement fractions, and that the results ran, as a rule, parallel.

TABLE I.

The Effect of Adding Fresh Guinea Pig End-Piece to Guinea Pig Mid-Piece Removed from Complement Inactivated by Distilled Water.

Five per Cent. Sheep Corpuscles, 10 Amboceptor Units; Complement Fractions Obtained by N/250 Hydrochloric Acid. Total Volume in Each Tube 5 c.c. Incubation, 1 Hour and 30 Minutes.

Fresh end-piece.	Distilled water mid-piece.	Hemolysis.
0.2 c.c.	1.0 c.c.	Complete
0.2 c.c.	0.5 c.c.	"
0.2 c.c.	0.3 c.c.	"
0.2 c.c.	0.2 c.c.	"
0.2 c.c.	0.1 c.c.	"
0.2 c.c.	0.06 c.c.	Almost complete
0.2 c.c.	0.04 c.c.	Strong
0.2 c.c.	0.02 c.c.	Little
0.2 c.c.	—	0
—	2.0 c.c.	0
—	1.0 c.c.	0

It is evident, therefore, that although distilled water serum as a whole may show no complement activity, yet on the addition of fresh end-piece to its globulin fraction, lively hemolysis may occur.

What, now, is the fate of the albumin fraction (of the toxophore group or end-piece) of the distilled water complement? Table II indicates the complement activity (1) of fresh guinea pig serum, (2) of guinea pig serum incubated in physiological salt solution

(dilution 1 : 10) for one and one quarter hours in a water bath at 37° C., and (3) of guinea pig serum incubated for the same period in distilled water (dilution 1 : 10).

TABLE II.

Ten Amboceptor Units, 5 per Cent. Sheep Corpuscles; Total Volume in Each Tube 4 c.c. Incubation Time of I and II, 2 Hours; of III, 6 Hours.

Guinea pig serum.	Hemolysis.		
	I	II	III
0.2 c.c.	Complete	Complete	Faint trace
0.1 c.c.	"	"	0
0.08 c.c.	"	"	0
0.06 c.c.	"	"	0
0.04 c.c.	Almost complete	Almost complete	0
0.02 c.c.	Little	Little	0
0.01 c.c.	Slight trace	Slight trace	0
0.004 c.c.	0	0	0

It will be seen that although the serum incubated in physiological salt solution has retained its original complement activity intact, when incubated in distilled water, on the other hand, its complement activity has become practically nil. The question now arises: Can toxophore activity be demonstrated in such inactive serum (table III)?

TABLE III.

The Effect of Adding Distilled Water Albumin Fraction to Fresh Globulin Fraction.

Fractions Obtained by Carbon Dioxid. Globulin Fraction Washed Three Times in Distilled Water. Five per Cent. Sheep Corpuscles, 10 Amboceptor Units; Total Volume in Each Tube 4 c.c. Incubation Time, 3 Hours in Water Bath at 37° C. Maximum Hemolysis at the End of One Hour.

Distilled water guinea pig end-piece.	Fresh guinea pig mid-piece.	Hemolysis.
0.1 c.c.	1.0 c.c.	Very strong
0.1 c.c.	0.5 c.c.	"
0.1 c.c.	0.3 c.c.	"
0.1 c.c.	0.2 c.c.	Almost complete
0.1 c.c.	0.1 c.c.	Complete
0.1 c.c.	0.08 c.c.	Almost complete
0.1 c.c.	0.06 c.c.	Strong
0.1 c.c.	0.04 c.c.	Moderate
0.1 c.c.	0.02 c.c.	Little
0.3 c.c.	—	0
0.2 c.c.	—	0
0.1 c.c.	—	0
—	1.0 c.c.	0
—	0.5 c.c.	0
—	0.3 c.c.	0

We see, therefore, that not only may the combination of distilled water globulin fraction + fresh albumin fraction display typical complement activity, but, what is far more striking, that hemolysis may also occur in the combination of distilled water albumin fraction + fresh globulin fraction.

Any attempt to explain these phenomena would be futile in the light of our present knowledge. The question becomes at once greatly complicated by these facts: (1) that all efforts to produce hemolysis by combining the distilled water albumin fraction with the distilled water globulin fraction, even in the most varied quantitative ratios, have met with failure; and (2) that sheep globulin fraction, though retaining a completing action for fresh guinea pig end-piece under a variety of conditions (*i. e.*, after heating at 55° C. for one half hour, after incubation in distilled water at 37° C., etc.), yet produces not the slightest degree of hemolysis when added to distilled water guinea pig end-piece. That summation action can not be the essential explanation of these phenomena is demonstrated, not only by table III but by the following protocol (table IV) as well, in which diminishing amounts of distilled water end-piece are combined with fresh mid-piece in the ratio of 1:1, 1:2, and 1:4.

TABLE IV.

Distilled water end-piece.	Fresh mid-piece.			Hemolysis.		
	I	II	III	I	II	III
1.0 c.c.	0.1 c.c.	0.2 c.c.	0.4 c.c.	Almost complete	Almost complete	Almost complete
0.08 c.c.	0.08 c.c.	0.16 c.c.	0.32 c.c.	Very strong	Very strong	Very strong
0.06 c.c.	0.06 c.c.	0.12 c.c.	0.24 c.c.	Moderate	Moderate	Strong
0.04 c.c.	0.04 c.c.	0.08 c.c.	0.16 c.c.	Little	Little	Moderate
0.02 c.c.	0.02 c.c.	0.04 c.c.	0.08 c.c.	Slight trace	Slight trace	Trace
0.01 c.c.	0.01 c.c.	0.02 c.c.	0.04 c.c.	0	0	0
0.3 c.c.	—	—	—	Slightest trace	Slightest trace	Slightest trace
0.2 c.c.	—	—	—	0	0	0
0.1 c.c.	—	—	—	0	0	0
—	1.0 c.c.	1.0 c.c.	1.0 c.c.	0	0	0

A further observation deserves brief mention, although it has no particular bearing upon this work. It was reported several months[4] ago in a study of quantitative relationships between fresh guinea pig end-piece and guinea pig mid-piece that, as a rule, hemolysis

[4] Marks, *Ztschr. f. Immunitätsforsch.*, 1910, viii, 508.

might be reduced or even entirely inhibited by increasing the quantity of globulin fraction over albumin fraction. Fränkel,[5] however, has recently reported an opposite effect; namely, an acceleration of hemolysis by an increase of the globulin fraction. These apparently contradictory results are to be ascribed to differences in technique. If the globulin fraction be used at once, no inhibitory effect can be detected. On the other hand, if it be allowed to stand under distilled water for a variable length of time so that it dissolves with some difficulty in physiological salt solution, reduction or inhibition of hemolysis is the rule, when the end-piece-mid-piece ratio of 1 : 1 is exceeded. Of further interest is the observation that although large quantities may inhibit hemolysis, nevertheless, the completing action of such globulin fraction may not infrequently extend as far as fresh globulin fraction.

[5] Fränkel, *Ztschr. f. Immunitätsforsch.*, 1911, viii, 781.

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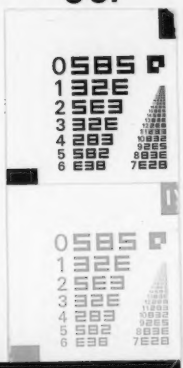
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